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SCIENZE AMBIENTALI
XXI CICLO

FROM CONSTRUCTION TO DIFFUSION OF KNOWLEDGE IN AGROECOLOGY

(AGR/02)

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Abstract

This doctoral project deals with the development of solutions to the need for actions, as suggested by the European Action Plan for Organic Food and Farming, to support the training and education of all stakeholders related to organic agriculture. In particular, 'From construction to diffusion of knowledge in Agroecology' is addressed to students, researchers and Institutions involved in the organic agriculture sector, and facing with weed control. In fact, among all plant production features, weed control still represents the bottle neck of the organic agriculture production system. For this reason, the work of thesis is made up and developed in two phases: a *construction phase* and a *diffusion phase*. The construction phase deals with the development of new solutions for organic weed control. The research has been centered on the possible use of essential oils for weed control in the phase of seed germination. Essential oils of lavender, cinnamon, peppermint and citronella, were tested in a scale up system on weed and crop seeds. The experiment was performed with controlled conditions (germination chamber) for temperature, humidity and light, as for the utilization of inert substrate such as filter paper into Petri dishes. These features allowed to check just the phytotoxic effect of compounds tested. In a second step, the research moved to semi controlled condition (green house). Light, humidity and the substrate utilized (loamy soil), made the experiment much closer to the natural environment which is characterized by more variability and biological activity and allowed to assess the real potentiality of the essential oils as bioherbicides. At the same time, experiments on the genotoxicity, bioconversion and biotransformation of essential oils were carried out. Results permitted to assert that essential oils are effective in reducing seed germination controlled conditions, while environmental factors and enzymatic and microbiological transformations make their application in the open field more difficult. Hence, further research is necessary to develop an appropriate technology of essential oils application. The diffusion phase could contribute to developing solutions to problems faced during the research. The diffusion phase, in fact, deals with the design and management of an E-Learning platform. The aim of an E-Learning platform is in this case to contributing to create a dynamic community of students, researchers and stakeholders with a focus on weed control in organic farming. This telematic "tool" can boost and improve communication and interaction between learners. In fact, while internet gives free

access to huge stores of information, E-Learning is structured and designed to support users in the task of turning information into knowledge. In this way, it should be possible to develop a common base of knowledge starting with research results and then promoting dialogue in learning groups to be extended to external and potential users, such as external networks of teachers and researchers in private institutions and agricultural Universities involved and willing to improve the organic agriculture sector either at national or international level.

INTRODUCTION

1.1 The evolution of communication and society

One of the most important things in our life is the ability to communicate with each other. Communication has evolved over millions of years through two main interaction modes: one involves co-located face-to-face communication and the other involves the use of sounds and signals alone in situation where line of sight or direct contact is hindered. Both communication modes connect synchronous communication which over millions of years evolved in complex speech. Hence complex speech has conferred evolutionary advantages (Kock, 2008). Today, the environment of communication network is changing. We communicate through media, body movement, speech, written and typed letters, and of course through computers, electronic mail and computer processed data. Speech continues to be the most important way of communication but, if people do not have the possibility to be in contact with each other and do not have the opportunity to communicate in synchronous way for geographical and time reasons, help is available with some forms of electronic devices. The rapidly growth of new communication device such as Internet, the widespread proliferation of mobile communications, and the global alliance of communication carriers, suggest that we are entering an advanced information age of true matter (Tanaka *et al*., 1998; Kock, 2008). In this view electronics and information technology can greatly improve the quality of life in the field of alternative communication. Technological changes in infrastructure for communication networks and information tools for human-to-machine interfacing are indispensable for diversified communication services. Even if as concepts, communication and information are very closely related, they also designate more than their usual conceptual meaning when they are called upon in social theories as well as in philosophical theories about the reality and the truth of social life; information and communication are then designating *physical events or event like objects* of the observable reality (Robillard, 2005). The communication and information environment is changing. The motivation for such big changes comes from society's needs. These needs observed in modern society represent major changes in the social framework, such as shifts from "centralized to dispersed," from "local to global," from "shared-use to personal-use," and from "monopoly to competition". These changes are very important for social progress, while resolving many current challenges, such as the aging of society, environmental problems, and globalization (Tanaka *et al*., 1998). If from one side technical improvement has been necessary for the growing needs of society, from the other side communication has to transform in order to match these changes. These changes depend on the agent's definition of the situation, and this is not solely a matter of subjective motivation. The meaning to which social action is oriented is primarily intersubjective meaning (Habermas, 1979) constitutive of the socio-cultural matrix in which individuals find themselves and act: inherited values and world views, institutionalized roles and social norms, and so on. Hence, the large use of the internet network is changing communication and the information distribution, following the evolution of society. Whether these changes are actually accomplished depends on the provisioning of technical innovation to meet these demands. The implementation of infrastructure technologies which support the basis of communication networks, such as transmission and exchange, has been promoted to match changes in social needs at good cost-performance, based on the evolution of computers, software equipment and information and communication technologies (Tanaka *et al*., 1998). If from one side computer and software development have been much more easier and characterized by the technological improvement, much effort has been done to improve communication in order to match, address and fulfill these technical improvements. Communication indeed had to adjust and adapt its form to the new information device. In this context of technological improvement followed by communication and social development, online learning (E-learning) is a tool largely wide spreading. *E-learning is associated with the information communication technology system and has the advantage of communicate and inform people in different geographical area and with different knowledge background without discrimination*. For accomplishing this target, communication has to be adapted at the different needs of people utilizing language and tools easily understandable and available such as participatory media and communication development.

1.1.1 Role of Information and Communication Technologies in society

Information and Communication Technologies (ICT) is a general term referring to the practical applications within the field of socio-economic development. ICTs can be applied either in the direct sense, where their use directly benefits the disadvantaged population in some manner, or in an indirect sense, where the ICTs assist organizations or non-governmental organizations or governments in order to improve socio-economic conditions. Hence communication and society are developing together influencing each other. In this view the development of communication can be simply characterized as the use of communication to promote development. More specifically, it refers to the practice of systematically applying the processes, strategies, and principles of communication to bring about positive social change. The first definition of development communication given by Quebral (2001) sentenced as *"the art and science of human communication linked to a society's planned transformation from a state of poverty to one of dynamic socio-economic growth that makes for greater equity and the larger unfolding of individual potential"*. The development communication becomes an important catalyst for change. For instance, local folk media are employed to reduce media's prejudice toward literacy and provide information in a traditional and familiar form. Development journalism provides people with information on change in their society, and works at the local level to advocate change. Development communication is characterized by conceptual flexibility and diversity of communication techniques used to address the problem. Some approaches to solve problems include: information dissemination and education, behavior change, social marketing, social mobilization, media advocacy, communication for social change, and participatory development communication. In all these forms the easy accessibility and the role that anyone can have editing information represents a crucial role. Consequently are acquiring an important position the participatory media.

1.1.2 Participatory media

Participatory media include (but aren't limited to) blogs, wikis, RSS, tagging and social bookmarking, music-photo-video sharing, mashups, podcasts, projects and videoblogs. Participatory media represents the real innovation, the backbone of the development in communication and participatory communication. Once given the accessibility and freedom to share information at all the levels and to all the people with different background, participatory media have become a tool to compare and improve knowledge. Participatory media convey the common intent of actively involving people who are the ‘subjects’ of development in shaping the process. In particular participatory media are social media whose value and power derive from the active participation of many people. In this way social networks amplified by information and communication networks improve the transparency. Transparency has to be framed, clarified and addressed in the human meaning implying openness, communication, and accountability. Furthermore, transparency with the meaning of holding public officials accountable and fighting corruption of information. These are psychological and social characteristic. Hence sharing knowledge is about freedom. It is dealing with the five freedoms identified by the economist Amartya Sen (1999) in “Development as Freedom”, as key for human and social development: *political freedoms; social opportunities* (such as education); *transparency guarantees* (as a right of every citizen); *protective security* against risks (such as ill health); and *economic facilities* leading to greater autonomy. In most cases, this development is where similarity ends and a diversity of differences begins. People's participation became defined in many different ways and this in turn led to numerous unresolved disagreements. Generally, four different ways of participation can be observed in most development projects claiming to be participatory in nature (Uphoff, 1985). They are:

1. *Participation in implementation*: people are actively encouraged and mobilized to take part in the actualization of projects. They are given certain responsibilities and set certain tasks or required to contribute specified resources.
2. *Participation in evaluation*: leading the achievement of a project where people are invited to critique the success or failure of it.
3. *Participation in benefit*: people take part in enjoying the result of a project.
4. *Participation in decision-making*: people initiate, discuss, conceptualize and plan activities they will all do as a community.

Some development initiatives provide people with opportunities to all these four ways of participation, while many do not, and restrict participation to one or two ways. It is a common agreement that participation in decision-making is the most important form because gives people control of both their lives and the environment and results that have to be achieved. Participation in decision-making deals with the improvement of the decisional process (see chapter 1.3). Applying this method, people acquire problem solving skills and full ownership of the result they want to gain. These are important elements which will contribute towards securing the sustained development of the community. Although supporters of participation appreciate more good than bad in the approach, they recognize at the same time that there are some limits. An international conference of practitioners and researchers working in participatory communication announced three cautions (White, 1994) at the end of their meeting:

1. Participatory communication processes are not a solution for development. Such processes are not suitable for solving all problems in all contexts or time frames.
2. The apparently opposing concepts of participation and manipulation can be viewed from many perspectives. The interventionist who attempts to "sell" solutions to target problem may be accused of being manipulative and may also be bringing along a whole set of alien cultural premises.
3. The price that people have to pay for taking part in participatory processes is often ignored. It is often assumed that people have nothing better to do. For every hour spent participating there is an opportunity cost; that is, the fact that people may have more productive activity if the participatory process does not lead to benefits, either in the long or short term.

Hence for better planning communication development these key points should be taken into consideration. First of all, the communicator figure has to be set. The communicator should be recognized by the community and his/her duties should also ideally be defined. To be congruent with the goals of participatory communication, *communication should be an instrument to empower the people rather than a vehicle for moving information*. In this context communication for facilitating actions should aim at a number of objectives:

- Creating a very clear understanding of the proposed action.
- Gathering feedback to determine if the course of action is acceptable and supported by (ideally) all; and if not to discover the preferred alternatives.
- Communicating the finalized course of action.
- Providing support and appropriate publicity as the action is being implemented.
- Keeping members informed of progress and the gathering of their reactions.
- Reporting the impact of the action.
- Assembling and sharing members' reactions to the action taken.

Participatory communication, in the ideal situation, is practiced spontaneously by people without mediation. The mediation is coming out from the participatory process itself. In this way communication finishes to be the simple transfer of information. The question of who initiated a communication, how decisions are made leading-up to the communication became more important than what is communicated. Communicators are no longer neutral movers of information but are intervening actively to activate changes aimed at encouraging people's participation. In many ways the techniques of communication are not changed. What changes profoundly is the ideologies and philosophies behind the practice of the techniques. The emphasis on interpersonal and traditional methods encourage the development and use of these communication methods. Participatory mass media organizations usually have some form of strong audience research mechanism. This may not always be in the form of a formal or scientific research unit managed by trained social scientists. The real effectiveness relays in bringing and overwhelming the gap between scientific knowledge and the average citizen. In this way it is possible to inform and communicate people about social problems in a direct and simple way. The benefit relays in involving the average citizen in the decision making process. The feedback, and the feed-forward, comes through people interaction with media workers sharing their views. Feed-forward become more important than feedback among some media workers. Feedback is when people react to stories or programs conceived independently by the media workers. Feed-forward is when people tell the media workers what is important for media coverage, and which is the best approach and way of covering these topics. By participating, people are claiming power for themselves, so threatening the influence of the power-holders. Conflict also frequently occurs among the people. The community is sometimes split into fractions by disagreements over goals and methods of doing things, and the involvement or exclusion of certain members of the community. Participatory communication which sets out to address original causes of development tends to cause high conflict. This history of conflict has caused many practitioners to appreciate the need for equipping themselves and the people with conflict managing skills. The most important of these are skills for negotiation and mediation. Participatory programs often threaten the interests of power-holders who may then retaliate against the people taking part in such programs. Facilitators should be mindful of such risks and explain them to the people who should then make their own decisions on the amount of risk they are willing to stand as a group. Many practitioners have tried to draw upon experiences from a number of other disciplines in addition to development communication. In the process they have contributed towards the start of a long overdue convergence of experiences in the education, communication and development sectors. Many development communication practitioners and researchers believe that participatory communication is the most appropriate theory to guide work and improvement in society. The most important future of development communication is its feature of sustainability. In this frame a sustainable development has the advantage of maximizing the use of available resources in order to ensure the long-term security of present and future beneficiaries. Hence sustainable development is a continuous progress which aims for and maintains a constructive state of living in society as preserved by social institutions and systems. Community people participation is a voluntary involvement of an informed and motivated community while being equipped with proper knowledge and training in which they are equally gratified. It is the active involvement of members of a particular social unit in all aspects of developmental procedures (planning, decision-making, evaluating, monitoring, etc...).

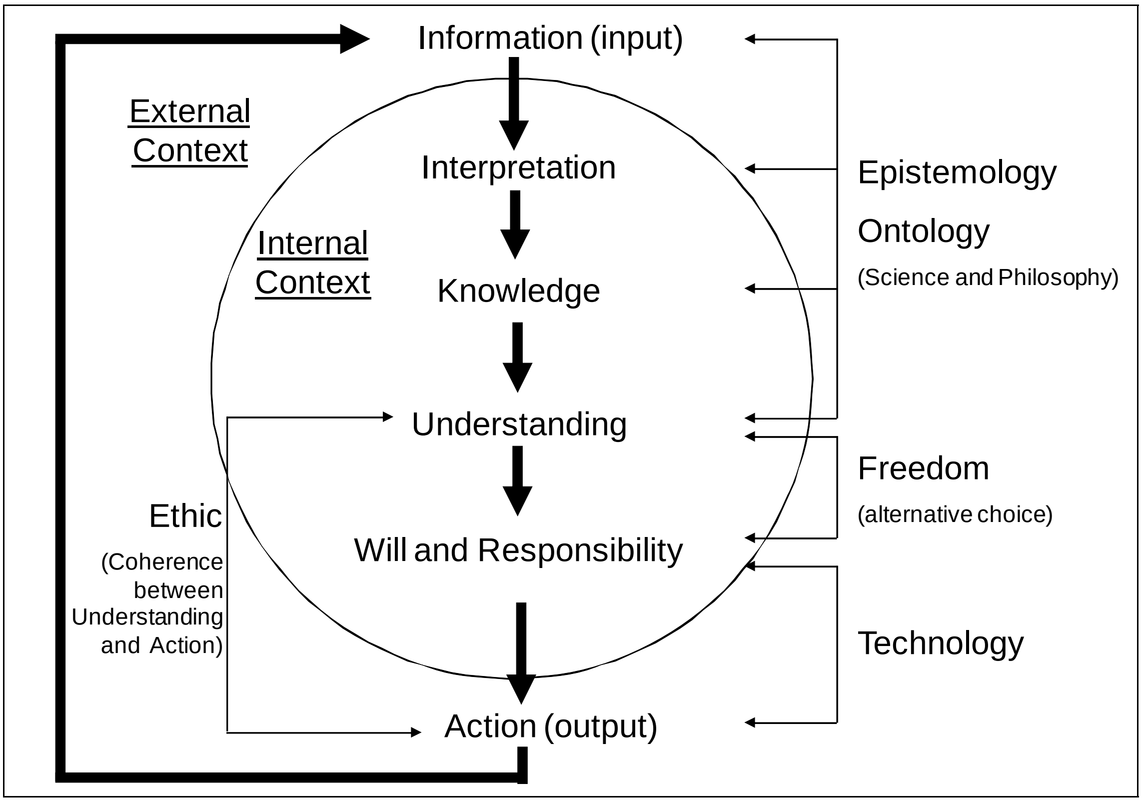
1.2 Philosophy of communication

Ontology is the study of the nature of being, of existence, or of reality in general and of its basic categories and their relations. There is an interrelationship of social ontology, social knowledge and information base for the description of social concepts and individuals (like persons, agents and organizations, and their relationships). Special terms consisting of names from social ontology then identify the so-called social contexts for the contextualization and optionally the fusion of semantically heterogeneous statements. As concepts, communication and information are very closely related, but they gain additional meaning when used in the context of social or philosophical theories about the reality and truth of social life (Robillard, 2005). Robillard (2005) states that, in the procedural ontologization of information, the words information and communication are used to designate *physical events* or *event-like objects* of the observable reality. Hence it is possible to speak about 'informational ontology' intended not into the concept of 'formal ontology' as used in cognitive sciences, but as a categorization technique whose purpose is to represent a set of knowledge propositional contents in structure similar or associated with knowledge-based system technologies. The concept of information is often subjected to a dramatic meaning shift, from epistemic to ontological, rendering it unsuitable for giving an oversimplified view of social facts. Communication, as a concept, is self-referential: it means what it means only because it logically implies that it is a right representation of a special order of social factuality (Gilbert, 1992). The evidence of the informational character of the behaviors included under the joining category of “social behavior” is hence not even contestable. Social behavior is behavior, because is directed towards society, or taking place between members of the same species. The thesis is that sociality is about a communication process involving selection of information visualized as an immaterial element of sociality. The main difficulty with this view is that it does not seem possible to define this concept of information in a legitimate way that should be significant of social facts, actions or events that are not explainable in informational terms (Robillard, 2005). Language cannot cover the entire range of sociality. A linguistic approach of sociality would necessarily not clearly distinguish between the level of the fact, or the action, or the event as it can be described, and the level of its linguistic proper description. Sociality is what characterizes the individual consciousness of the moral norm imposed to individuals by society and in spite of the individual’s will and his or her ethics of the norm. The social level is from that point of view a supra-individual order. This duality is easy to identify in the majority of sociological textbooks in any language. The concept of social, consequently, refers to the order of society’s constitutive structure in opposition to the order represented by the concept of sociality. Sociality, in fact, describes the ways by which people interact and connect with each others, and where communication is only one of them. Hence, a general theory of communication cannot pretend to offer a full-length portrait of what “society” is like, because this is just not where communication amongst socialized people can be detected (Robillard, 2005). A general theory of communication cannot consequently emerge as a justification matrix for a general theory of society that is called in its turn just to serve as an interpretative matrix. This does not mean that a general theory of society cannot integrate concepts like “communication” in its theoretical structure, rather it would have to apply a general circumspection to its usage. To begin, this theory should be based on the empirical observation that when people communicate, and they effectively do so by means of language and/or other symbolic devices such as texts, images, etc., what they communicate to others is not information, but <u>meanings</u>. Understanding sociality is therefore beginning to understand what role linguistic communication plays in binding individuals together. The linguistic communication takes place in a context. These circumstances play a great part in how linguistic and non-linguistic expressions are formulated and carried out. Therefore, linguistic communication takes place in defined contexts and is about <u>sharing meanings</u>; consequently, context determines that meanings are in a self-reliant means, in the sense that they cannot be communicated in forms other than in linguistic ones and they refer to what connects individuals in the context of the very linguistic communication. What we need, then, is a model of social communication that integrates a model of sociality as partly describable in communicational and linguistic terms. What we need is a theory of social meaning, not a semantic, but a theory that would show how it is possible for meanings to be socially effective in a pragmatic way. Such a theory could handle systemic concepts and means, but would not need to base its epistemological thesis on any information theory whatsoever. Meaning and information are not comparable, from a pure informational point of view. It would leave systemic concepts work as they should be working, within the analyticity and abstraction level they belong to. Social communication can therefore be labeled a systemic type of communication, meaning that it concerns abstract properties and variables describing a world of meaning that only exists because of its usefulness to procure safe theoretical explanations about what could not otherwise be apprehended (Robillard, 2005). It gives a conceptual idea for understanding some set of social facts, actions or events whose elements escape direct observation. It is not a set of rule to understand the information distribution in society; for this is a conceptual flaw. For analyzing and understanding any object, it is not important to consider the vision created merely in a specific moment, but the picture as consequence of the past and future evolution. Within this approach, the holistic vision of the input/output system analysis instead of the reductionist one is right epistemology. The equilibrium has to be reached in an extended horizontal time and not in a specific moment. The sustainability of the system is taken into consideration within the holistic approach. For a sustainable process, a systemic approach is indispensable. When a systemic approach is applied, the system is stable and long-lasting. Sustainable development is a continuous progress which aims for and maintains a constructive state of living in society as preserved by social institutions and systems.

1.3 Improving decisional process through E-Learning

The decisional process is the expression of the human culture as concretization and coherence between thinking and action. Furthermore is the consuetudinary act giving man the ability to actively modify the environment in which he lives. Every man and every institution (coordinated group of people) are regarded as an internal context operating into an external context, i.e. a given biophysical and socio-economic environment (Figure 1).

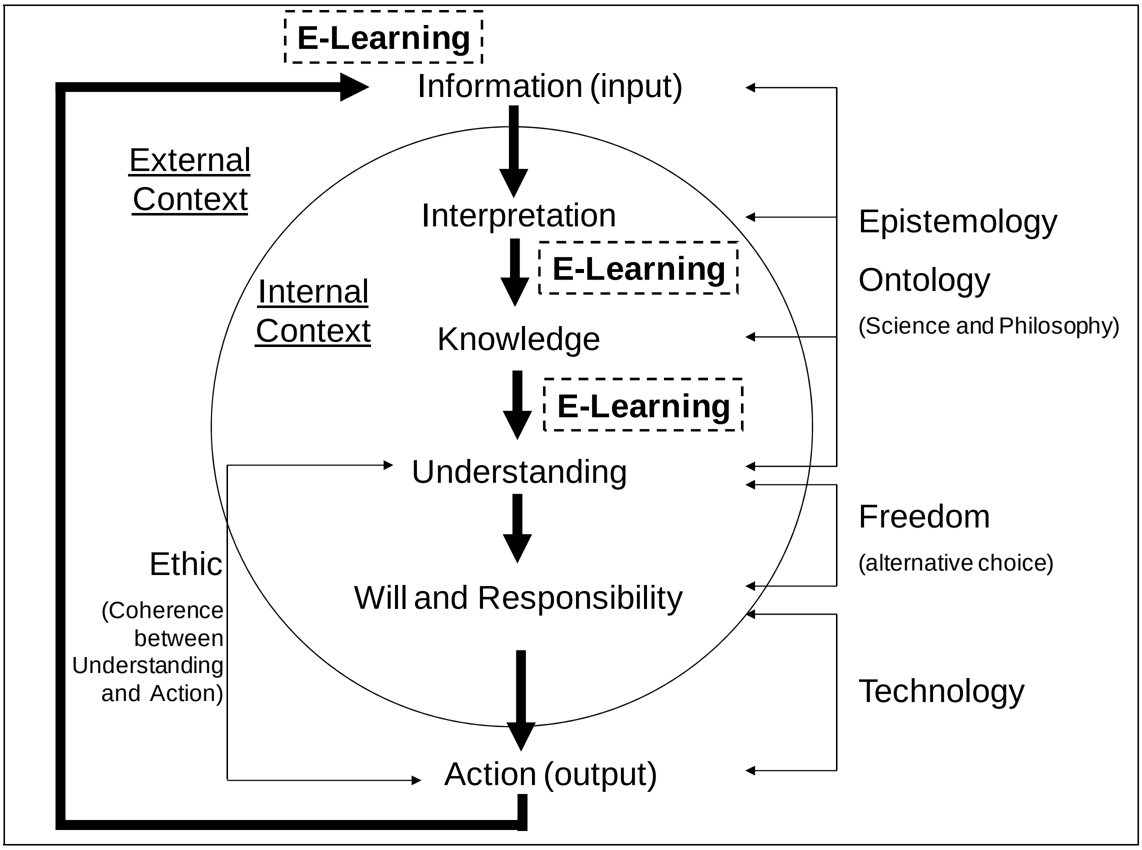
Figure 1 Decisional Process (Modified from Caporali, 2005)



What is inside the borderline (internal context) is taken into consideration and analyzed as a whole applying an holistic approach and a soft systems methodology (Checkland and Scholes, 1990). In this way it is not a reductionist approach but just an enquiry at a different level of hierarchy. The aim of the model is to represent and explain the reality through an indivisible interconnection of parts giving a rich picture of the whole situation. The rich picture representing the whole subject of investigation has in this way a value higher than the sum of each single component present in it. The reason is in the intrinsic and extrinsic features fitting together as a whole in opposition to the reductionist vision largely applied in scientific research (Bawden, 1991). This modified context and this approach give new information for the further decisional cycle. The approach is holistic. The emphasis is pointed on the relations between components as a whole process in a space-time perspective instead of the merely analysis of the single components. Hence the systemic approach produces new knowledge that balances time and space dimension taking into consideration the cause, the consequence and the improvement of utilizing scientific research. The process starts from and ends into the given context (Figure 1) in which man and institutions (coordinated group of people) operate receiving information (input) which are elaborated and modified (output). The relationship between environment and man is cyclic and the length of the process is relaying with the persistence of the person within the context. Hence the scenario is build up in co-evolution between man and environment. All the steps bridging the transformation of information into action are embodied by individual action within the context, but are the result of the previous decisional process as well. In the flow chart the crucial point is the interpretation of the sensorial data and their coordination and representation in what is defined as knowledge. Knowledge is the ability to build up real representative models in order to appreciate conscientiously in its entirety the relationships of the components within the environment in which we are living. The knowledge coming out from this process has to be further evaluated and judged to create understanding. Understanding is the ability and capability to apply knowledge in life. The whole process bridging information to understanding represents the traditional research identified as hermeneutic in philosophy and cognition in sciences. The following process bridging understanding to action is investigated by ethics. Ethics, encompassing behavior and style of life, puts into relation the whole decisional process with the incoming effect generated within the environment in which we are living. In the ethics phase, the representative ability of critical self reflection conveys at the highest rank of involvement bridging the freedom of choice among optional alternative and the willingness to execute it. This process embodies responsibility and awareness. The output of such a process is a new epistemology which represents reality as based on hierarchy and emergence giving also birth to inventiveness and creativity. Hence systemic knowledge has high explicative effect being integrative, ethical, and revolutionary and much more humanely acceptable.

In this framework, E-Learning plays a crucial role for improving the decisional process. Already in the interface external context/internal context, E-Learning provides much more accessibility and availability of information (Figure 2). There is a wider range of information in the electronic network than in conventional networks. These information represents the input for the internal context. Here the interpretation is facilitated by the E-Learning platform which gives birth to a community aimed at building up knowledge.

Figure 2 Decisional Process improved by E-Learning (Modified from Caporali, 2005)

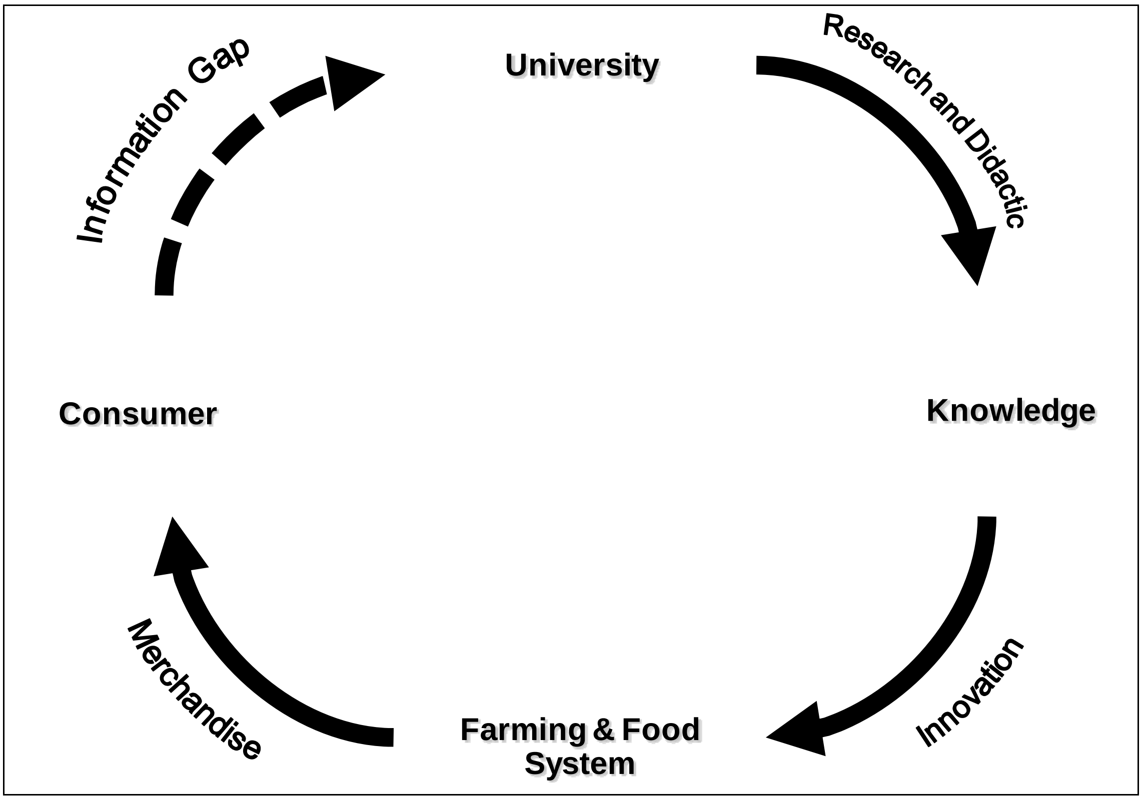


This community creates knowledge through information exchange and shared interpretation. It has already been demonstrated how E-Learning and E-Collaboration can improve the knowledge creation (Kock, 2008). This knowledge has to be evaluated and judged to create understanding. This process is made feasible through a participatory media process. Participatory Media include (but are not limited to) blogs, wikis, RSS, tagging and social bookmarking, music-photo-video sharing, podcasts. Participatory media convey the common intent of actively involving people who are the 'subjects' of development in shaping the process. In this way social networks amplified by information and communication networks improve transparency. Information generated by participatory media processes is as much or even more accurate than that generated by controlled processes (Giles, 2005). That because in the perfect situation, participatory media are practiced spontaneously by people without mediation. The mediation is coming out from the process itself. In this way, communication finishes to be the simple transfer of information becoming constructivism.

1.4 *Transferring of what and how*

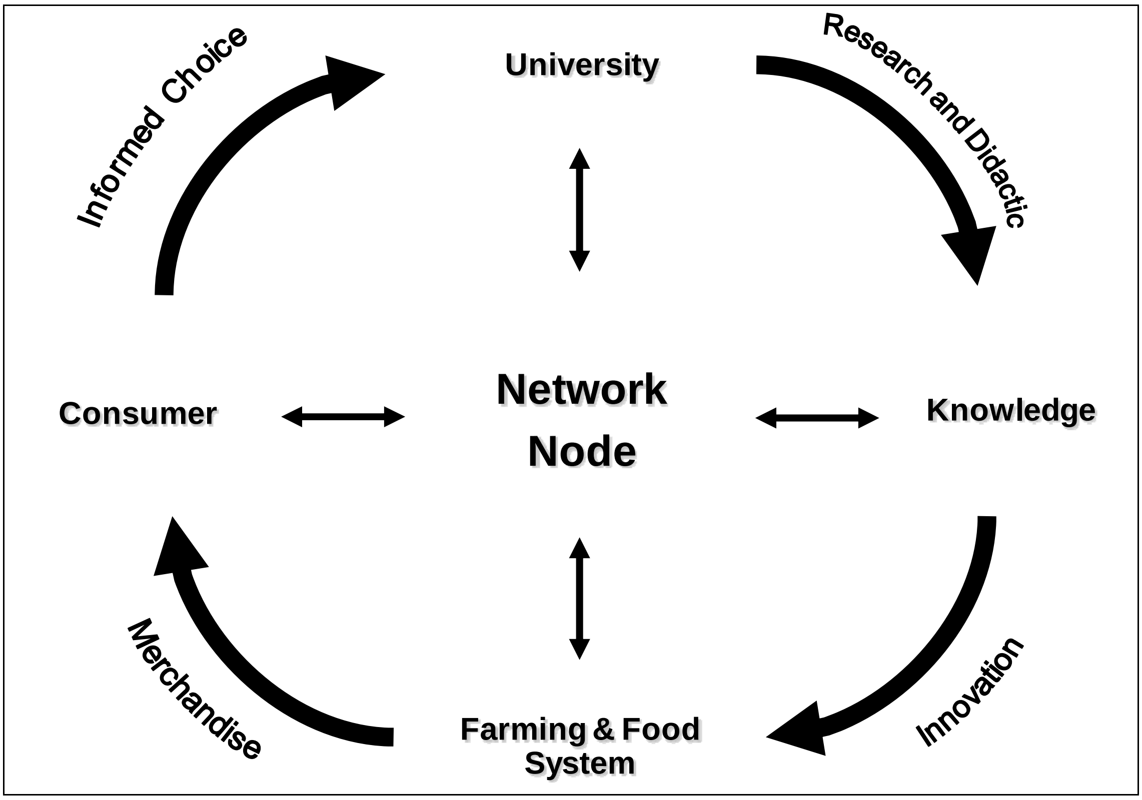
Academics define information as a set of data, facts, and figures that have been processed in such a way for becoming meaningful (Mchombu, 2004). When information is applied to do something globally related, it is said to have become knowledge. These definitions serve as useful purpose, when we consider information and knowledge from the point of view of formal education. The construction of knowledge is addressed at the improvement and security of humanity. Knowledge is not only produced by experts, but is the product of human creation. Since the beginning, human beings have been making discoveries. They learned to make and use the first stone tools, to domestic animals, and to grow plants for their needs. Even if the knowledge acquirement is not relaying at a single class or category of people, great emphasis is played by the academic world. Universities are the higher source of knowledge construction. The Magna Charta of the European University state that the University is a structure that "produces knowledge through education and research". Unfortunately, if the knowledge produced is not made available and accessible to everyone, produces few benefits. Most of the innovations made remain confined to few people. Their diffusion is made accessible only to experts throughout education, seminars, conferences or scientific journals and heading for a format public. Especially those who should benefit most, the “average citizen”, more often remain isolated. For this reason, it should be tried to develop and improve the diffusion path with the involvement of the largest number of people not implicated in the sector. One of the channel of information which in recent years is becoming increasingly spread is Internet. His accessibility and ease of use does not discriminate in any way the availability of information. These information are set up for developing and improving the society facing with changes and transformation. Especially the agricultural and agri-business sector has undergone rapid and significant change, particularly within the last twenty years. Economic, technological, regulatory, social, and climatic factors continue to shape and redefine this fundamental and intrinsic part of the economy. Rapid change in any economic sector usually necessitates the acquisition of new skills, new knowledge, and new practices. Training and life-long learning are therefore seen as key complementary imperatives. Unfortunately, the knowledge generated by Universities is seldom transferred directly to citizens or consumers. The knowledge flow usually arrives to the consumer step by step and being transformed as "un-understandable and un-modifiable choice" (Figure 3).

Figure 3 Information flow in the actual agriculture system



A way of improving consumer awareness of and involvement in decisions to invest in organic agriculture and in research related to it could be the development of an E-Learning platform (a node in a network) aimed at diffusing information about the organic agriculture. E-learning is a general term used to refer to computer-enhanced learning. Citizens are influenced by the information spread by mass media, but have little say in what they are presented. An E-Learning platform would give them the opportunity to become more active, choose the information they find interesting, and make suggestions to farmers and researchers, thereby influencing the market supply of organic foods. In this way, the platform could represent a direct channel between the diverse actors involved in the system, binding the citizen with the policy and decision makers (Figure 4). The platform could give information about the progresses made in agricultural research, making them accessible to the average citizen and allowing them to make more responsible choices. The usual information flow starts from University, where knowledge is produced through research and didactic. The outcome becomes available to the agricultural industry and sector by way of innovations for the market system. Innovations generate new products available to the average citizen.

Figure 4 **E-Learning as a way to improve information flow for organic agriculture**



Products are received by consumer that can only express their satisfaction and try to influence the research activities for new products by buying or not buying the new products. With the creation of an E-Learning platform, there could be the opportunity of creating a link between all the different actors appearing in this process. The advantage is in the interaction rising from the direct communication of all the diverse actors. Each one of them could influence the others by giving and receiving feedback in an effort to improve the organic agriculture framework, facilitating the achievement of social awareness, while relying on economic, social and productive sustainability.

1.5 Thesis Object

In the 1970s and 80s interest in and consumption of organically grown agricultural products were restricted to small niche markets, with produce usually supplied locally. However, in the last two decades, stakeholders' perception of food has greatly changed, focusing more and more on food safety, environmentally friendly and ethically sound production (Codron *et al*., 2006). In the mid 1990s, a series of food safety crises, such as mad cow, blue tongue, and foot and mouth diseases, as well as avian flu, and workers' rights scandals relating to the working conditions in factories in developing countries, have raised awareness in consumers, as well as society's, about environmental and ethical issues related to agricultural production (Codron *et al*., 2006). Consumers started to pay more attention to what they consume and the repercussions of their consumption habits. Although there is generally some knowledge and awareness about organic agriculture, consumers are not conscious of what organic products really are and what is involved in producing them. Even worst, the many sources of sometimes misleading information appear to overwhelm today's consumers, giving competing and sometimes conflicting signals that only help to confuse consumers. In fact, even if consumers understand the broad issues regarding organic foods, many of them tend not to understand the complexities and soundness of organic farming practices and quality attributes of organic food. In particular, the environment and social attributes of organic agriculture are lost to them, and other features such as health and food safety are also not easily conveyed nor are they easily defined. To support the growth of the organic production sector and increase consumers' awareness of its benefits, it is important to communicate how environmental and social issues are dealt with in organic agriculture (Wheeler, 2008). Aside from vague ideas about not using pesticides in production, most consumers have no idea of what the differences between organic and conventional foods are. This is due to a lack of reliable, available and accessible information. Basically, all the information on organic agriculture is produced for the main actors involved in the farming and food system chain. Furthermore, research output is confined to scientific journals which, aside for being difficult and expensive to access, are written in scientific jargon, which is hard for the average citizen to understand. As stated in the "Magna Charta Universitatum", which describes fundamental values and rights of Universities, the University is a structure that both produces and should spread knowledge to younger generations. This knowledge should be accessible to everybody, especially the average citizen, who is the main stakeholder when innovations brought about by research are implemented. In this light, the European Action Plan for Organic Food and Farming (EC, 2004) has identified the need for actions supporting the training and education of all stakeholders related to organic agriculture, covering aspects related to production, processing, marketing and their benefits.

Hence 'From construction to diffusion of knowledge in Agroecology' is addressed to students, researchers and Institutions involved in organic agriculture and particularly facing with weed control in organic agriculture. In fact, among plant production features, weed control still represents the bottle neck of the organic agriculture production system. For this reason, the work of this thesis is made up and developed in two phases: a *construction phase* and a *diffusion phase.* The construction phase deals with an attempt of constructing a knowledge base of new solutions for organic weed control, while the diffusion phase deals with the allocation and exchange of information within stakeholders involved in the same topic and research. In this way, this thesis aims at outlining a new pathway for transferring knowledge from research to stakeholders with the use of an E-Learning platform.

CONSTRUCTION PHASE

2.1 *Weed management*

Modern agriculture is the result of a long of development of new techniques for improving the production system. Since World War II, agriculture has traced a path from self-sufficient and regionally oriented farming to commercial and export-oriented agribusiness (Anaya, 1999). Unfortunately, this trend has been conditioned by the industrial and commercial infrastructure with its bare emphasis on the reductionist approach and technological fixing with menaces for the sustainability of the agroecosystem (Gliessman, 1983; Bentley, 1987). The disadvantage of conventional agriculture is the creation of too simple crop systems replacing the natural plant community, with a lost of soil fertility and biodiversity. The battle against weeds, pests and microorganisms, in fact, is addressed without considering some potential beneficial effect that they can have on crops and crop management. A common practice to tackle weed infestation is to use products obtained by chemical synthesis although herbicide resistance in weeds is rapidly expanding throughout the world with higher costs of production and greater environmental impact (Anaya, 1999; Lemerle *et al*. 2001). Inevitably, this practice has caused both ecological and environmental problems or risk for human health (Wyse, 1994; Buhler, 1996; Caporali, 1991, 2004). For this reason, in the last years more and more emphasis has been put on finding out new and alternative solutions to weed control and pest management. Research on traditional management of crop pests has mainly focused on a more rational use of natural components and processes of the agroecosystem providing the chance for more appropriate technologies of sustainable production without damaging the environment (Anaya, 1999). “Weeds have traditionally been seen as an enemy” in the agriculture sector, representing one the main reason of yield decrease (Lampkin, 1990). Many weeds, approximately 250 weeds species, are considered to be problematic in agriculture (Worsham, 1989). Their effect in the agriculture production can be quantifiable to a lost of 1/3 of the total production, due to the competitive factors (Oerke *et al.* 1994). The new approaches in weed management, therefore, consists of the integration of direct weed control with other cultural practices such as crop and cultivar choice, tillage, fertilization, etc.. Integrated Weed Management System (IWMS) is recognized as the best approach to tackling weed problems (Bàrberi, 2001). Weed control is even more important in organic agriculture, where herbicide use is forbidden. In this case, agronomic choices have crucial impact on weed management, and, if appropriate, are capable of great advantages in weed control.

Plants compete with each other's for light, water and nutritive elements; therefore, the potential inherent ability to directly inhibit competitors by mean of allelochemical activity is an important goal to investigate (Overland, 1966; White *et al.* 1989; Bradow and Connick, 1990), especially for developing a new approach to weed management based on the utilization of natural defenses of plant species. Among natural defenses, there are mainly agronomic choices making one crop species much more competitive on the others. One of the feature coming out from competitive ability is the developing of a larger canopy as result of a faster growth and a better use of resources or inhibition of the competitive ability of weeds.

2.2 Allelopathy

The term allelopathy is derived from the Greek words allenon (of each other) and phatos (to suffer) and refers to injurious effect of one plant upon another (Molish, 1937; Rizvi *et al.* 1992). Allelopathy as a phenomenon has been widely reviewed (Molisch, 1937; Rice, 1984; Duke and Abbas, 1995; Dukes *et al*. 1996, 1997, 1998).

Allelopathy is defined as "the direct or indirect effect of one plant upon another through the production of chemical compounds that escape into the environment" (Rice, 1974). Another definition given by the International Allelopathy Society (1996) states that allelopathy refers to "any process that involves secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological systems". This second definition underlines the allelopathic effect as exerted from all components present in the biological system instead of just plant. This feature gives a wider spectrum of action to allelopathy, even if the interaction between plant is the most important for weed control.

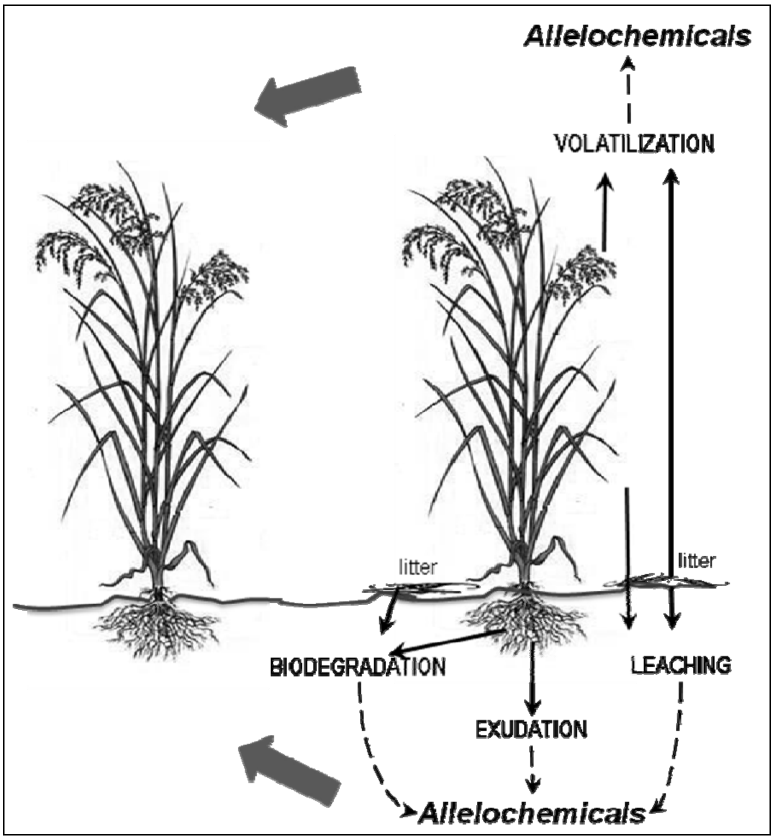
Compounds released from plants and imposing allelopathic influences are defined allelochemicals (Putnam and Tang, 1986; Kruse *et al*. 2000). Consequently, allelopathy refers to chemical interactions taking place between plants through the release of allelochemicals. This effect is exerted by the joint action of several compounds and not only by a singular compound, whose quantity and persistence in the environment may be not sufficient to obtain a toxic response, so allelopathic activity in a plant community results from the combined effects of several compounds. However, there are some exceptions such as, for example, juglone from walnut, which have inhibitory effect as single compound (Rice, 1984). The effect could be either inhibitory or stimulatory, depending on the concentration of the compound (Inderjit and Keating, 2000). Many plants release a variety of compounds with allelopathic potential. They are essentially organic compounds exuded from the root system or released, through degradation, by crop residues or shoots (from leaves by volatilization and/or by leaching) (Weston, 1996; Inderjit and Keating, 2000). Once synthesized, these compounds are stored in vacuoles or intracellular spaces (Chou *et al.* 2003), in numerous parts of the plant including roots, rhizomes, leaves, stems, pollen, seeds and flowers (Devi *et al.* 1997; Kruse *et al*. 2000). These compounds are secondary metabolites produced by plants in response to biotic or abiotic factors, (Rice, 1984; Einhellig, 1996, 1999). Allelopathy offers potential for a biological weed management trough the production and release of allelochemicals, and its use has been well documented in literature (Putnam, 1988; An *et al.* 1998). The study of the allelopathy effect is the first step for investigating plants reaction and defense by means of secondary metabolites which, when identified, could represent the constituents of new herbicides. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. The function or importance of these compounds to the organism is usually of ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition. The success of many pharmaceuticals of biological origin has been dependent on this fact. Therefore natural products as guide for plant growth regulators are a strategy that have to be investigated for the discovery of new herbicides (Duke *et al.* 1999). The present understanding of the biochemistry, morphology and chemistry of natural products has given new chances to this kind of studies (Putnam and Duke, 1978; Einhellig and Leather, 1988). Plants containing volatile allelochemicals have frequently been described for this potential (Sigmund, 1924; Went, 1942; Bonner, 1950; Muller, 1986). In particular, Muller *et al*. (1964) reported that in the surrounding of species such as aromatic shrubs there are no annual plants in a diameter of 90 cm and the presence of annuals weed is very limited within 2-6 m. Anyway, the allelopathic potential of many plants is increased by exposure to various environmental stresses (Einhellig, 1987, 1996) and induces phytochemical variation (Kong *et al*. 2002). Most secondary metabolites have yet to be discovered, and relatively few natural compounds have been examined for a broad range of biological activities, especially phitotoxicity.

2.2.1 Action of essential oils as allelochemicals for weed control

Allelopathy influences/effects other organisms only when the allelochemicals produced by the plant are released into the environment and in surrounding area of other plants (Lui and Lovett, 1993). Weidenhamer (1996) states that the amounts of compounds released seems to be a function of the biomass and density of the source plant, as well as of the concentration and solubility of the specific allelochemical.

Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment (Figure 5) by root exudation, leaching from aboveground parts, and volatilization and/or by decomposition of plant material (Rice 1984; Devi *et al.* 1997).

Figure 5 **Release of allelochemicals by the donor to the target plant (modified from Devi *et al.* 1997)**



Mainly, while volatilization and leaching are limited to some genera of plants, root exudation and decomposition of plant residues are the most common ways of action of the allelochemicals compounds released (Devi *et al.* 1997). When susceptible plants are exposed to allelochemicals, germination, growth and development may be affected. The most frequent reported gross morphological effects on plants are inhibited or retarded seed germination, effects on coleoptile elongation and on radicle, shoot and root development.

2.3 Screening of allelochemicals for weed control

Research and technology focused on the discovery of natural active principles as bioherbicides are not different from those concerning biologically active molecules of synthetic pesticides or pharmaceuticals. One of the main investigation used for discovery allelopathic compounds for agriculture crops has been that one of screening a large numbers of crop plants and natural vegetation for their aptitude to suppress weeds (Duke *et al*. 1999). The first action towards this goal is the identification of allelochemicals of plant origin, whit their mechanisms of production and persistence in the environment. Bioassays in the lab on plant extracts are commonly screened for checking their effect on seed germination. After that, trials in the greenhouse and in the field have to be performed for verifying the effect on plant development (Cornish *et al.* 1993; Gauvrit & Cabanne, 1993; Dudai *et al*. 1999). In this way the allelochemicals provide the basic structure for developing new synthetic herbicides. At the same time, the allelochemical itself could be used as a valuable “natural” herbicide. What is required is the necessary examination and experimentation of these compounds. In this view, research has focused mainly on ways to take advantage of natural derived chemicals to enhance crop production and develop a more sustainable agriculture having less impact on the environment and on human health. The inhibitory effect, anyway, depends on the species from which the essential oil is extracted (Dudai *et al*. 1999). Essential oils are generally classified “as safe” and are also able to inhibit the growth and abundance of microorganisms in food (Ismaiel & Pierson, 1990; Wilson *et al.* 1997; Beuchat, 2001). They may be used as a feasible weed control tools in organic farming systems, but more information on phytotoxicity is necessary before implementing field experiments (Tworksoki, 2002). One of the main aspects that have to be taken into consideration is the “mixture effect” of the compounds released (Kruse *et al*. 2000). The identification of an active allelochemical compound does not mean that this is the only compound involved in allelopathy. Einhellig (1995) states that an allelopathic effect often is the result of the combined outcome of several compounds. Laboratory experiments indicate that mixture solutions of allelochemicals have greater effect than the same concentrations of the compounds used separately (Blum *et al.* 1999; Einhellig, 1995; Chaves & Escudo, 1997). Experiments have indicated that the mixture of some allelochemicals can have allelopathic activity even if the concentration of each individual compound is significantly below its inhibitory level (Blum *et al.* 1993; Blum, 1996). In summary, what have to be investigated, is not the single compound able to act as bioherbicide, but the synergy played from all the compounds composing the allelochemical agent (i.e. Essential oil).Research and literature review have demonstrated that phytotoxic natural products act on a larger number of herbicides target sites (Duke *et al.* 1999). That is why the pesticide industry is focusing more and more its attention on the secondary metabolite produced naturally from plants. Among the secondary metabolites observed, essential oils produced naturally from aromatic plants are ones which have more possibility of application.

2.4 Research developed

The research developed has been focused on the investigation of essential oils for weed control in the phase of seed germination. Essential oils of lavender, cinnamon, peppermint and citronella were tested on the germination of crops and weed seeds among the most common in the Mediterranean environment. Trials have been carried out in a scale up system. The experiment started from a situation of controlled environment (germination chamber) moving to semi controlled condition (green house). Phytotoxicity, genotoxicity, enzymatic and microorganism bioconversion taking place in the seeds during the germination process were the features explored.

2.5 Germination Chamber – Phytotoxicity of essential oils

The first screening for the possible use of essential oils as bioherbicides has been developed in a germination chamber of the Department of Plant Production at the University of Tuscia. The controlled conditions of temperature, humidity and light, as well as the utilization of inert substrate, such as filter paper into Petri dishes, allowed to check only the phytotoxic effect of compounds tested. In particular, the allelopathic effect of essential oils extracted from cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) was tested on seed germination of seven common weed species from the Mediterranean environment: *Amaranthus retroflexus* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Chenopodium album* L., *Sinapis arvensis* L., *Lolium* spp., and *Vicia sativa* L..

2.5.1 Materials and Methods

The germination tests were carried out in Petri dishes (10 cm diameter) in a germination chamber in the dark at controlled temperatures of 27°C during the day, and 15°C during the night for the spring-summer weeds, and 20°C and 10°C respectively for the autumn-winter weeds. The effect of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) essential oils on the weed seed germination rates was evaluated. The compounds were provided by Muller & Kostner. All the compounds were certified IFRA-RIFM-CEE 91/155 GMO's free and for cosmetic, pharmaceutical and herbalist use. Seeds of some of the most common weed species responsible for high yield loss and crop interference present in the Mediterranean environments both in winter and summer crops were selected. The weeds included in the study were redroot pigweed (*Amaranthus retroflexus* L.), black nightshade (*Solanum nigrum* L.), common purslane (*Portulaca oleracea* L.) and common lambsquarter (*Chenopodium album* L.) for the spring-summer cycle, and wild mustard (*Sinapis arvensis* L.), ryegrass (*Lolium* spp.), and common vetch (*Vicia sativa* L.) for the autumn-winter cycle. Before starting the test, all seeds were placed in a germination chamber at a temperature of 5°C in the dark for two days, to break dormancy and synchronize germination. For each essential oil, an oil-in-water emulsion was prepared at a concentration of 0.2, 0.6, 1.8 and 5.4 mg l^{-1} referred to as C1, C2, C3 and C4, respectively. The essential oils utilized were 100% pure. The pH of the solutions assayed is shown in table 1. Each Petri dish contained 25 weed seeds placed on two layers of filter paper (Whatman® No.5) wetted with 5 ml of oil-in-water emulsion (area of 79 cm^2). The control dishes (C0) were wetted with distilled water instead of an oil-in-water emulsion. The control treatments also served to calculate the maximum germination rates of weed seeds used. The Petri dishes were sealed with laboratory film (Parafilm® M) in order to permit the exchange of oxygen and CO_2 but not the volatilization of the essential oil (De Feo *et al*. 2002). Each treatment, including control, was replicated four time. Seedling emergence was recorded on a daily basis until the control stabilized, reaching the maximum germination rate. Radicle elongation has previously been used as a measure of inhibition activity (Dudai *et al*. 1999). In fact, even if a seed produces a radicle, it does not mean that the seed can germinate completely and develop the coleoptile. The coleoptile growth and the ability of the seed to develop into a plant is, in fact, more reliable than the radicle germination (ISTA, 2004). Thus, in this study, coleoptile elongation was used as a parameter of inhibition of germination. Relative germination was calculated as the percent germination of the treatment compared to the control. For statistical evaluation, data were transformed when necessary to arcsin $[x\ (\%)/100]^{1/2}$ (Gomez and Gomez, 1984). ANOVA was employed to test for significant differences between means of each seed weed species and each essential oil concentration. For each weed seed species a simple linear regression analysis was used to study the correlation between percent seed germination and essential oil concentrations. For each essential oil the regression lines of all weed species were compared using the analysis of variance as suggested in (Camussi *et al*. 1986). For all statistical analysis, the SAS program was used (SAS, 1993).

2.5.2 Results and Discussion

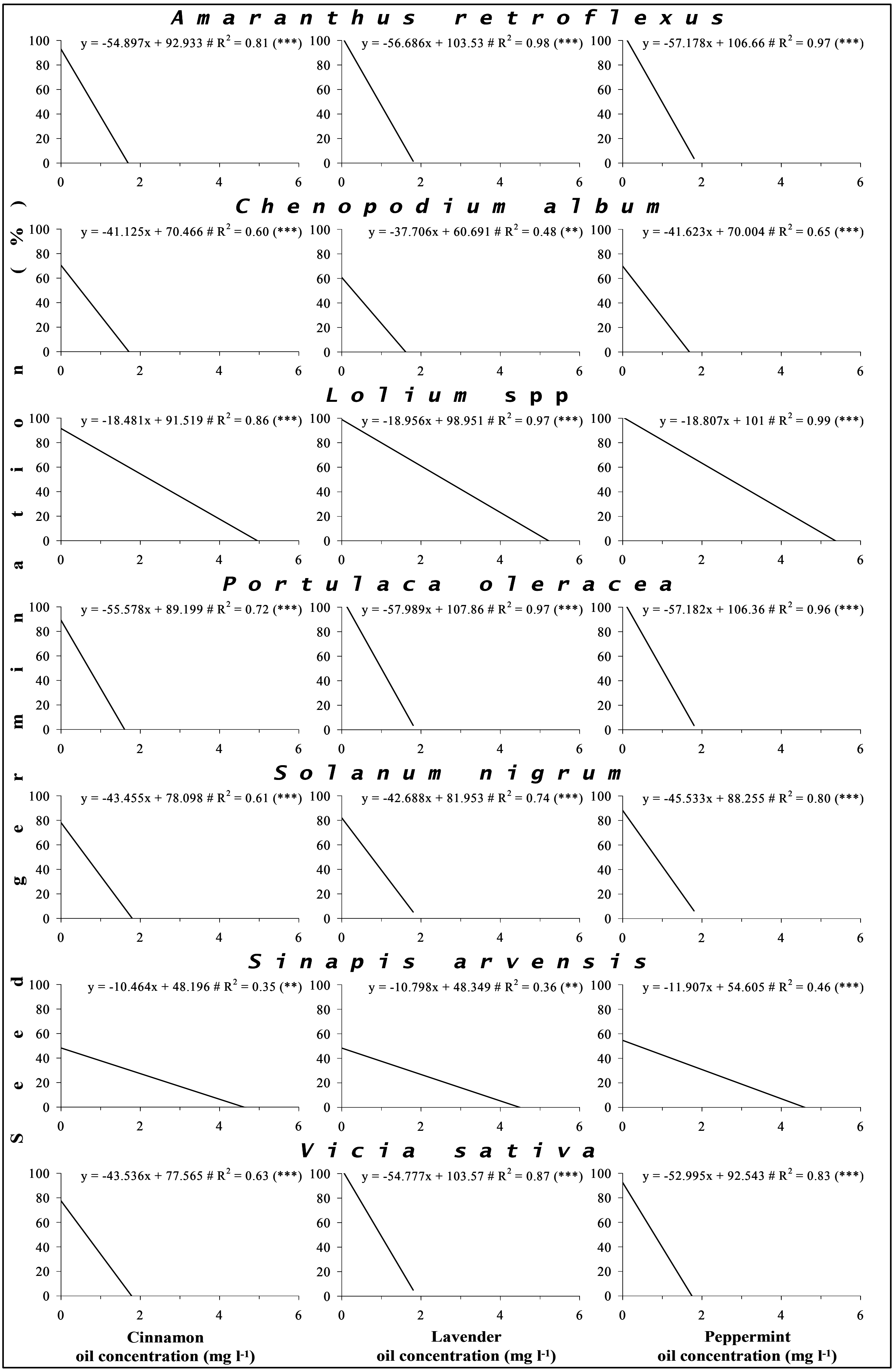
The results confirmed that essential oils inhibited the germination of weeds. The lethal dose able to completely inhibit weed seed germination was different for each of the essential oils studied, though various tolerances for different oil concentrations were exhibited by the weed species (Table 1). Among the oils tested, cinnamon generally exerted the highest inhibitory effect, compared to lavender and peppermint. Generally, the germination rate decreased with increasing concentrations of essential oils even if a dose-dependent relation between oil concentrations and inhibitory ability was not always observed. The weed species most sensitive to the essential oils were redroot pigweed (*Amaranthus retroflexus* L.), common purslane (*Portulaca oleracea* L.) and common vetch (*Vicia sativa* L.), their germination being completely inhibited by all oils at concentration C3. Common lambsquarter (*Chenopodium album* L.) and black nightshade (*Solanum nigrum* L.) were inhibited at C3 by cinnamon oil and at C4 by lavender and peppermint essential oils. Wild mustard (*Sinapis arvensis* L.) and ryegrass (*Lolium* spp.) were the least susceptible species, only being completely inhibited at the highest oil concentration (C4) (Table 1). Even if a lethal concentration for all the weeds was identified, the sensitivity of the various species to the oils was quite different.

Table 1 **Inhibition percentage of weed species in response to the concentrations (C1 – C4) of the oils tested compared to the control (C0)**

Weeds	Cinnamon essential oil					Lavender essential oil					Peppermint essential oil				
	C1	C2	C3	C4	LSD P≤0.05	C1	C2	C3	C4	LSD ≤0.05	C1	C2	C3	C4	LSD P≤0.05
	%					%					%				
Amaranthus	11	60	100	100	*23*	7	26	100	100	10	6	16	100	100	9
Chenopodiu.	71	54	100	100	*8*	80	77	99	100	7	61	70	97	100	8
Lolium	3	19	68	100	*11*	2	7	47	100	6	2	8	35	100	5
Portulaca	6	82	100	100	*16*	2	17	100	100	6	6	17	100	100	7
Sinapis	80	76	87	100	*7*	74	79	92	100	7	66	67	89	100	7
Solanum	63	38	100	100	*14*	51	39	94	100	13	40	31	94	100	14
Vicia	62	41	100	100	*19*	17	11	100	100	19	21	47	100	100	20
LSD (P≤0.05)	*18*	*21*	*7*	–		*15*	*12*	*8*	–		*14*	*14*	*8*	–	

At C1 a strong inhibitory effect was observed for wild mustard (80% inhibition by cinnamon, 74% by lavender and 66% by peppermint essential oil) followed by common lambsquarter (71%, 80% and 61% by cinnamon, lavender and peppermint, respectively) and black nightshade (63%, 51% and 40% for cinnamon, lavender and peppermint, respectively) (Table 1). The effects of oils at concentration C2 was erratic. For instance, common lambsquarter was inhibited more by cinnamon and lavender oils at concentrations C1 than at C2 while peppermint essential oils inhibited lambsquarter germination more at C2 than at C1. The regression lines between seed germination and essential oil concentrations confirm the different susceptibility of the weed species (Figure 6).

Figure 6 **Regression lines of seed germination and oil concentration in seven weed species treated with three essential oils**



While the inhibition effectiveness is similar among oils, weed species differ in their response to the toxic effect of each oil. Table 2 displays the significant differences between the regression lines for each essential oil. It appears that the concentration of essentials oils has a greater effect on weed susceptibility than the type of oil used.

Table 2 **Analysis of variance of the comparison among the regression lines of seed germination of seven weed species treated with different concentration of cinnamon, lavender, and peppermint essential oils**

Cinnamon oil				
Effect	DF	MS	F Value	Significance
Model	13	10592.1	17.7	<0.001
Error	106	597.6		
Total	119	1689.4		
R-square: 0.68				
Non parallelism of curves	6	6455.0	10.8	<0.001
Unequal intercept	6	2751.6	4.6	<0.001
Non similarity of curves	12	4643.3	7.8	<0.001
Lavender oil				
Effect	DF	MS	F Value	Significance
Model	13	12873.3	34.3	<0.001
Error	106	375.5		
Total	119	1740.8		
R-square: 0.81				
Non parallelism of curves	6	7316.2	19.5	<0.001
Unequal intercept	6	5713.2	15.2	<0.001
Non similarity of curves	12	6171.1	16.4	<0.001
Peppermint oil				
Effect	DF	MS	F Value	Significance
Model	13	12566.3	42.7	<0.001
Error	106	294.1		
Total	119	1634.7		
R-square: 0.84				
Non parallelism of curves	6	7178.1	24.4	<0.001
Unequal intercept	6	4076.0	13.9	<0.001
Non similarity of curves	12	5689.3	19.3	<0.001

2.5.3 Conclusion

The results demonstrated that cinnamon, lavender and peppermint essential oils have a strong phytotoxic activity towards weed seed germination and can therefore be regarded as a potential source of bioherbicides. The regression analysis between seed germination and oil concentration (Figure 6) provides evidence that essential oils are equivalent in their phytotoxic effect while weeds differ greatly in their susceptibility to oil concentrations. The data, in fact, show that the inhibition ability does not differ among oil types: it is essentially dependent on concentration. The mechanisms by which essential oils inhibit seed germination remains largely unknown. Essential oils from cinnamon (*Cinamommum zeylanicum* L.) and red thyme (*Thymus vulgaris* L.) inhibit potato sprout growth by killing meristematic cells (Vaughn, 1991). Genotoxicity tests (Comet assay and Micronuclei test) indicated that essential oils from lavender (*Lavandula* spp.), caused a significative DNA damage in *Vicia sativa* L. seedling growth (Boccia *et al*. 2007; Sturchio *et al*. 2007). After germination, there could be some biochemical process or mitotic interference due to the essential oils that inhibit the cotiledon growth. An important point highlighted by our study was that the relation between oil concentration and inhibitory ability was not always dose-dependent for all the species. In particular, this effect was noticed for red root pigweed and common lambsquarter, as noted in (Mao *et al*. 2004), as well as on the germination of common vetch and black nightshade (Table 1). The fact that the germination of redroot pigweed was inhibited by essential oils is important as this species is an economically important weed and has developed a resistance to herbicides with different modes of action (Owen, 2001). Using essential oils in an alternative weed management system appears to be possible. Our data suggest that the concentration of essential oils plays a pivotal role in their eventual field application. Germination rates of common lambsquarter, wild mustard and black nightshade were reduced by over 50% at relatively low concentrations of essential oils. This is useful information in an Integrated Weed Management System (IWMS) framework. The combined action of diverse weed control measure could make feasible an application of the essential oils in an IWMS. For example, the application of an essential oils at a low concentration could inhibit weed emergence long enough during in the early stages of crop growth for the crop canopy to develop. Further weed control could be done mechanically. Another aspect of interest for an IWMS is the low susceptibility of ryegrass compared with the others weeds tested. Ryegrass is a monocotyledonous species, while all the others weeds are dicotyledonous, a selective use at the lower concentration of the essential oils could be hypothesized. The identification of the lethal dose of essential oils could be useful in greenhouses and field conditions. However, additional work is required to determine the mechanism of herbicidal action and to develop a formulation that could be used under field conditions. Despite the common belief that essential oils are highly volatile and their persistence under field conditions would have to be improved, the feature that have to be taken under consideration is the bioconversion of such compounds by the environment and microorganisms. Under field conditions, while is not. Their effects on crop species will also have to be investigated. Further field research is necessary to develop an appropriate technology of essential oil application for the inhibition of weed seed germination and the effect of such application on crop plants.

2.6 Green House – Phytotoxicity of essential oils

After the results obtained in the germination chamber (chapther 2.5), the research moved to semi controlled conditions. The experiment was carried out in semi controlled environment, such as a green house in the experimental farm of the University of Tuscia. Light, humidity and the substrate utilized (loamy soil), made the experiment close to the natural conditions characterized by variability and biological activity. The experiment was designed to check the inhibition ability effect exerted by the essential oils of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) on seed germination of common weeds species of the Mediterranean environment: *Amaranthus retroflexus* L., *Sinapis arvensis* L., *Lolium* spp..

2.6.1 Materials and Methods

The research was carried out in plastic pots of 400 ml (10 cm diameter, 8 cm height) in greenhouse (controlled temperature 25°C during the day, and 15°C during the night) to test the effect of the inhibition ability of the essential oils of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) on the seed germination of some common weeds species present in the Mediterranean environment, such as redroot pigweed (*Amarantus retroflexus* L.), wild mustard (*Sinapis arvensis* L.) and ryegrass (*Lolium* spp.). To remove the dormancy and synchronized the germination, before starting the test, all the seeds were put into the germination chamber at a temperature of 5°C in the dark for two days. Before the trial, the soil was put into the oven at the temperature of 104 °C until constant weight. In each pot filled with 0.5 kg of loamy soil, 25 seeds of each weed were sown at a depth of 4 mm. For each essential oil, the oil-in-water emulsion was prepared at the concentration of 5.4, 21.6, 86.4 and 345.6 mg l^{-1} named as C1, C2, C3 and C4, respectively. The essential oils utilized were pure at 100%. All the compounds were certified IFRA-RIFM-CEE 91/155 GMO's free and for cosmetic, pharmaceutical and herbalist use. An emulsion of 5 ml was sprayed on the soil surface of each pot (area of 78 cm^2). A control utilizing only water was performed as a test for the maximum percentage of germination. Each treatment, including controls, was replicated three times. The essential oil-in-water emulsion was sprayed on the soil surface just after the sowing; the daily irrigation was made only with water gently sprayed on the soil until it got humid without water leaching from the pot. Every day since the sowing date for 4 weeks the seedling emergence was directly observed and recorded. It was considered as germinated a seed from which a radicle emerged. Relative germination was calculated as the germination of the treatment divided by that of the corresponding control. Analysis of variance was performed to evaluate the effect of essential oil concentrations adopting the ANOVA procedures, while a regression analysis was performed between seedling emergence and concentration using the SAS program.

2.6.2 Results and Discussion

The use of essential oils has generally determinated a reduction of the seed emergence compared with the control with pure water (C0) since the start of the trials. The reduction of the germination appeared noteworthy already at the minimum concentration (C1). Among the essential oils tested, cinnamon has exerted the higher inhibition ability with a reduction of the germination compared with the control of the 40%, 56% and 51% respectively for ryegrass, wild mustard and redroot pigweed (Figures 7 – 8 – 9). Inhibition ability has been observed anyway at the lower concentration of lavender (C1) and peppermint (C1) for redroot pigweed and wild mustard, while ryegrass resulted being less susceptible to these oils. The results showed that there is not always a dose-dependent relation between oil concentrations and inhibition ability. Using the C2 oil concentration has been observed the same trend exerted by the C1 (Figures 7 – 8 – 9). Increasing additionally the concentration (C3), instead, the inhibition activity generally raised. Compared with the control (C0), cinnamon oil inhibited almost totally redroot pigweed (91%), while ryegrass and wild mustard were inhibited for the 47% and 49%. Lavender oil inhibited ryegrass (51%), wild mustard (59%) and redroot pigweed (78%) following the same trend. Peppermint showed instead an unexplainable activity. At the C3 concentration it exerted the maximum inhibitory activity for ryegrass (62%), and wild mustard (47%), while inhibited redroot pigweed of the 73%. The highest concentration tested (C4) of cinnamon and lavender oils have controlled significantly all weeds.

Figure 7 **Effect of the essential oils on the seed germination of ryegrass. In each graph the bars indicate LSD ($P \leq 0.05$)**

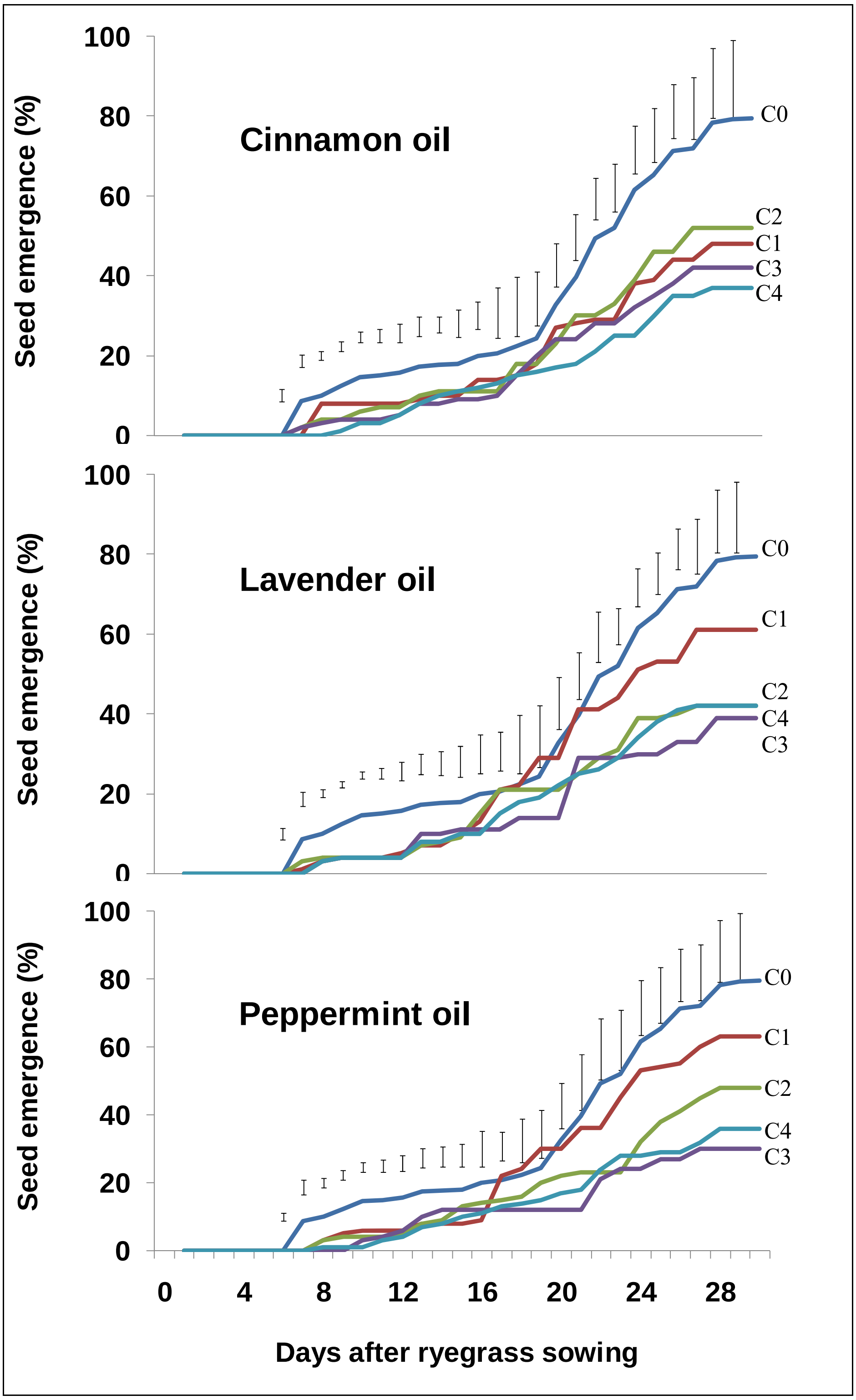


Figure 8 **Effect of the essential oils on the seed germination of wild mustard. In each graph the bars indicate LSD (P ≤ 0.05)**

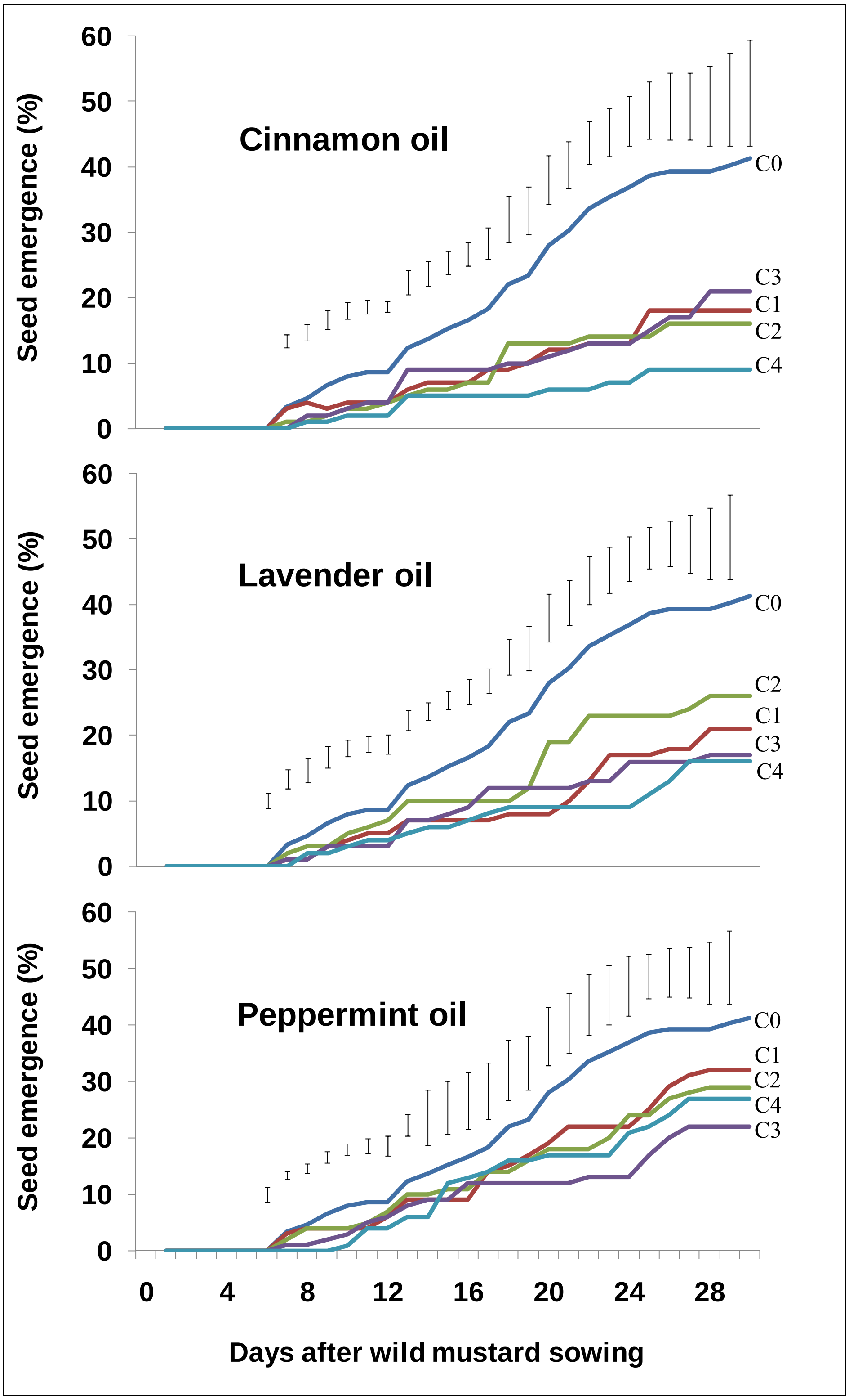


Figure 9 **Effect of the essential oils on the seed germination of redroot pigweed. In each graph the bars indicate LSD ($P \leq 0.05$)**

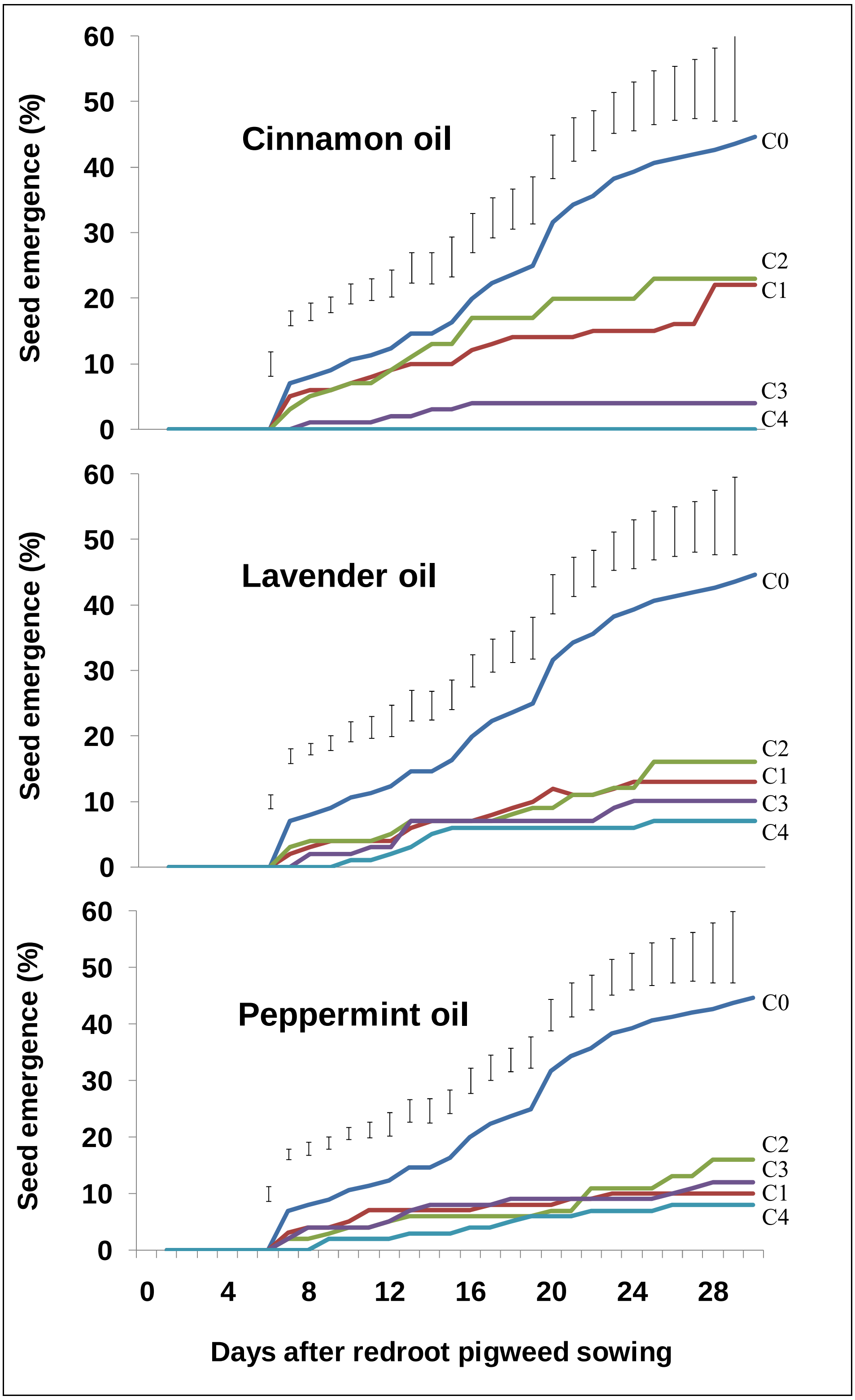
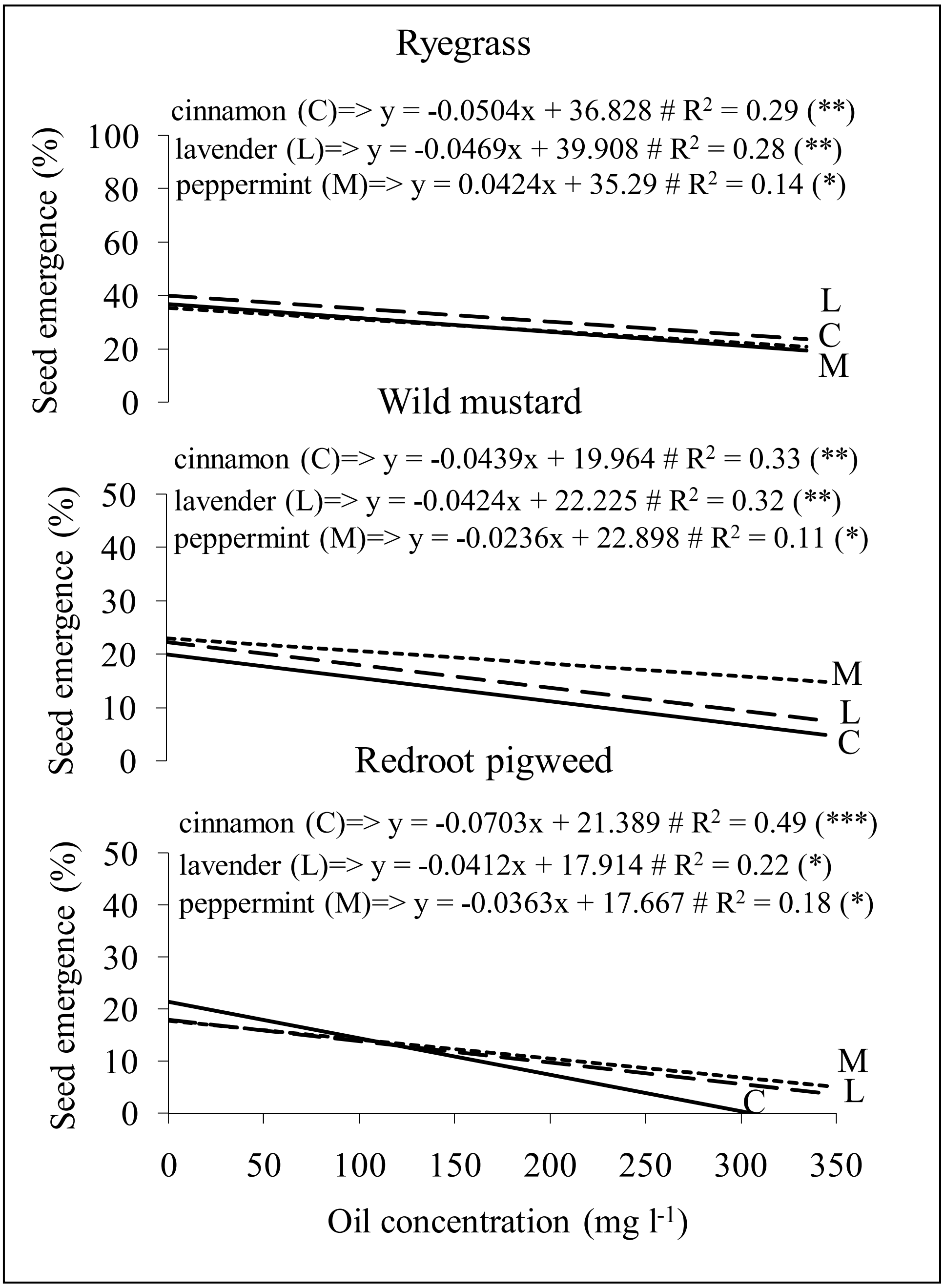


Figure 10 **Regression analysis between seed germination and essential oils concentration. *, **, *** = significant P≤0.05, P≤0.01, P≤0.001, respectively; ns = not significant**



Ryegrass was inhibited for the 53% by the cinnamon and 47% for lavender, wild mustard for the 78% of cinnamon and 61% of lavender, while redroot pigweed reached the lethal dose with cinnamon oil and the 84% with lavender. Peppermint instead showed higher inhibition ability only for redroot pigweed (82%), while for wild mustard (35%) and ryegrass (55%) the inhibition were lower than the C3 concentration.

The regression analysis between oil concentrations and seed germination shows as a general trend that increasing concentration of essential oil brings about increasing inhibitory effects on weed seed germination till reaching a lethal dose (Figure 10). It is possible to see how for wild mustard and redroot pigweed the cinnamon oil had the stronger inhibition effect, as shown by the regression coefficient values, while for ryegrass was the peppermint oil that showed a stronger tendency of inhibition effect.

2.6.3 Conclusion

This research shows that there is potential to control weed germination using natural compounds. Cinnamon is the oil with the stronger inhibition effect on the seed germination of redroot pigweed and wild mustard. The inhibition of redroot pigweed and wild mustard seedling emergence may have practical meaning because these species are economically important weeds and have developed a resistance in herbicides, even if with different modes of action (Owen, 2001). Peppermint oil has been the more effective in inhibiting ryegrass seed germination. The identification of this oil against a peculiar weed could be important in view of bioherbicides to be used with selectivity proprieties. So far, the results obtained make feasible to suggest an application of the essential oil as bioherbicide in controlled environment such as horticulture as in green houses. Persistence of essential oils should be improved, these natural compounds being highly volatile and so less lasting in the environment. On the other side, for the same reason they have short half time and are less harmful to the environment (Duke *et al.*, 1999). Further field research is necessary to develop an appropriate technology of essential oil application for inhibiting weed seed germination.

2.7 Germination Chamber – Genotoxicity of essential oils

After having proved the phytotoxicity of the essential oils (chapters 2.5, 2.6), a test on the genotoxicity was set out in collaboration with the Department of Plant Production and Anthropic Settlements of the National Institute of Health and Safety at Work (ISPESL-DIPIA). Aim of the research was the evaluation of the genotoxic effect of the essential oils as possible alternative to conventional herbicides. Indeed, the large and indiscriminate use of synthetic chemical herbicide for weed control has been often responsible for environmental problem and human risk. Herbicides are constituted by toxic substances, in some cases cancerogenous. The European Directives have contributed to reducing the risks of phytochemicals, establishing limits of their concentration in fruits, vegetables, cereals and animal products and regulating their classification, packaging, labeling, commercialization and use. Essential oils are generally regarded as safe by the United States of America Food and Drug Administration (FDA). Hence, in this research the essential oil of lavender (*Lavandula spp*.) genotoxic effect on *Vicia faba* seeds was evaluated.

2.7.1 Materials and Methods

50 *Vicia faba* seeds were sowed in 500 gr of sterilized sandy control soil in aluminium basins. Each basin containing 50 seeds, treated with water-emulsion of *Lavandula spp*. (120 ml of H_2O) at different concentrations (0, 0.2, 0.4, 0.6, 1.8, 5.4 mg l^{-1}) of oil, was allowed to germinate in climatic chamber at 20ºC ±1 for 5 days. A sandy soil basin, treated with 120 ml of water, was used as negative control. In effort to permit the exchange of oxygen and CO_2 but not the volatilization of the essential oil, each basin was sealed with laboratory film (Parafilm® M). The seedlings were used in the phytotoxicity and genotoxicity tests.

Phytotoxicity

The seedlings were taken out and the phytotoxicity was calculated by measuring the primary roots length of *Vicia faba* seedlings exposed to the essential oil to study the eventual toxic effects.

Genotoxicity

Micronuclei test: Micronuclei are Feulgen positive corpuscles localised within the cell wall in the cytoplasmatic area surrounding the main nucleus. They are formed by chromosome or chromosome fragments that are not incorporated into daughter nuclei at the time of cell division.

The genotoxic effects were evaluated by following the frequency of micronucleated cells (MC) in *Vicia faba* root meristems (De Marco *et al*. 1990). The micronucleated cells frequency was scored from 15.000 cells (12 root tips, 400-500 cells for tip).

The *Vicia faba* root tips were cut and fixed in glacial acetic acid and ethanol 3:1 (v/v) solution for 24 hours, therefore were treated with HCl 1N to 60°C for 8 minutes, dyed to Shiff reagent for 24 hours and fixed on microscope cover glass. For each samples were observed 6 tips to the optical microscope. The data were analyzed by Analysis of Variance (ANOVA) with a Dunnett's multiple comparison versus the control group, using the statistical software SPSS 6.1 (SPSS Inc.) Chicago, IL.

Comet assay: The Single Cell Gel Electrophoresis (SCGE) is a cytogenetic test to evaluate the genetic damage in single cell induced by mutagenic agents. It is a simple, sensitive and rapid short-term genotoxicity test; theoretically it can be applied to all type of cells, including vegetal cells. It's a method for measuring DNA ds/ss breaks, alkali-labile sites (abasic sites, oxidized bases etc…), that change DNA structural conformation, (only under alkaline conditions $pH \geq 13.5$). The test was performed under alkaline unwinding/alkaline electrophoresis (A/A) protocol Angelis *et al*. modified. The *Vicia faba* seedlings root tips were chopped in 0.5 ml of PBS with 10mM Na_2 EDTA. The suspension was filtered (20μm filter) to remove most of the tissue debris. The filtrate was mixed with 200 μl agarose of LMP agarosio to 0.5% in PBS. The microscope slides were pre-treated with a NMP agarose layer to 1% and a NMP second layer (150 μl) to 0.5-0.6%. The slides were dipped in lysis solution (2.5 M NaCl, 100 mM Na2 EDTA, 10 mM Tris-HCl and 1%TritonX-100 and 10% of DMSO, ph=10 added before to use) for 1 hour to 4°C. Then the slides were placed in the electrophoresis buffer applying 300 mA and 30 V for 40 min. After electrophoresis the slides were dipped in a neutralization buffer (0.4M Tris-HCl, pH=7.5) to RT for 15 min. The slides were therefore fixed (a first step in 70° ethanol and second step in absolute ethanol), rinsed in distilled water and dyed with 100 μl ethidium bromide (20 $\mu g\ ml^{-1}$) for 7 min. The comets were viewed by epifluorescence microscope with an excitation filter of 515-560 nm and barrier filter of 590 nm. The DNA migration was determined

using an images analysis system (IAS 2000, Delta arranges). For each comet several parameters was acquired. The % DNA was used as a parameter of DNA damage (µm).
A computerized image analysis system (IAS 2000 Delta Sistemi, Italia) was employed.The data were analyzed by ANOVA with a Dunnett's multiple comparison versus the control group using the statistical software SPSS (Chicago, IL).

2.7.2 *Results and Discussion*

The result showed a phytotoxicity effect of the essential oil at all concentrations tested (Figure 11). The inhibition ability of the essential oil on the primary root elongation compared with the control, was significative already at the lower concentration (0.2 mg l^{-1}). The genotoxicity tests - comet assay and micronuclei test (Figures 12 – 13), showed that the concentrations 1.8 - 5.4 mg l^{-1} of lavender essential oil caused a significative DNA damage, while at lower concentrations (0.2, 0.4, 0.6 mg l^{-1}) the comet assay results (Figure 14) showed a DNA damage increment only at 0.2 mg l^{-1} concentration (Sturchio *et al.* 2007). There is not a dose-dependent relation between oil concentrations and genotoxic effect.

Figure 11 **Phytotoxicity was calculated by measuring the roots length of *Vicia faba* seedlings exposed to the essential oil. An asterisk show significant differences with P<0.05**

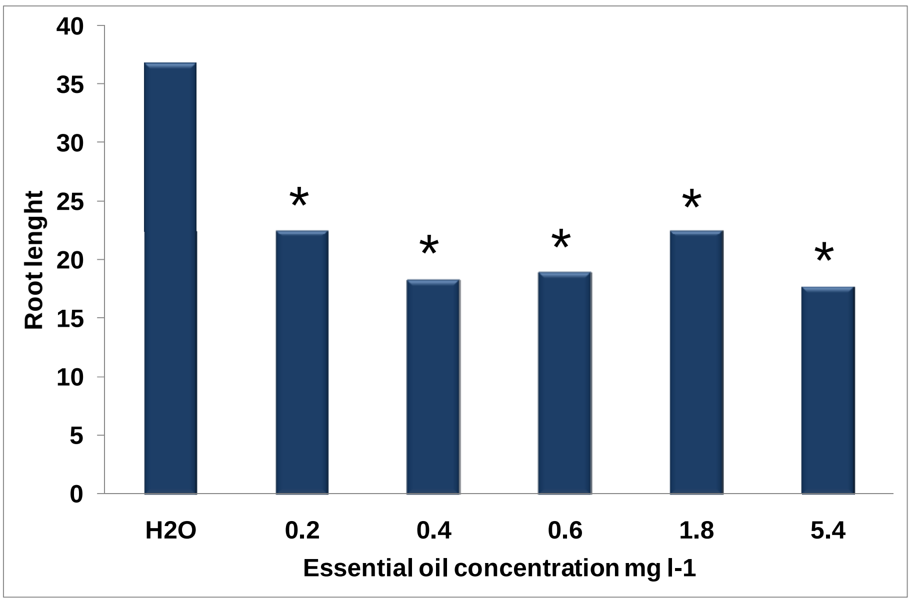


Figure 12 **Frequency of micro nucleated *Vicia faba* cell exposed to essential oil. An asterisk show significant differences with P<0.05**

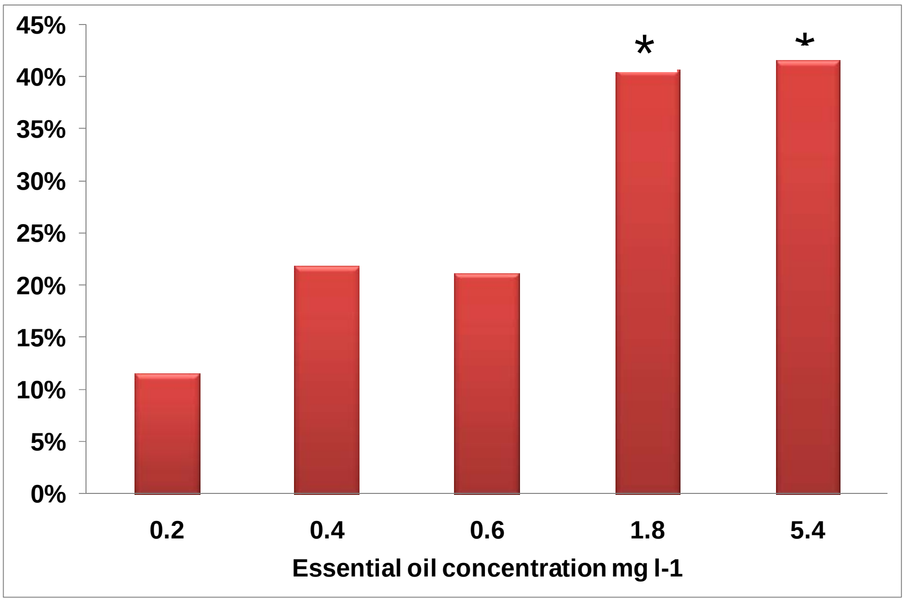


Figure 13 **Comet test on *Vicia faba* cells An asterisk show significant differences with P<0.05**

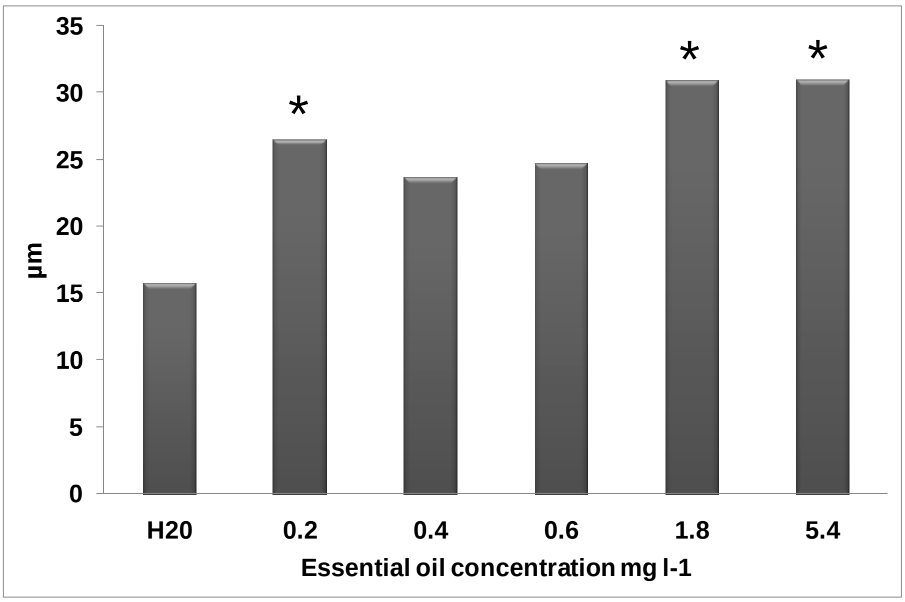
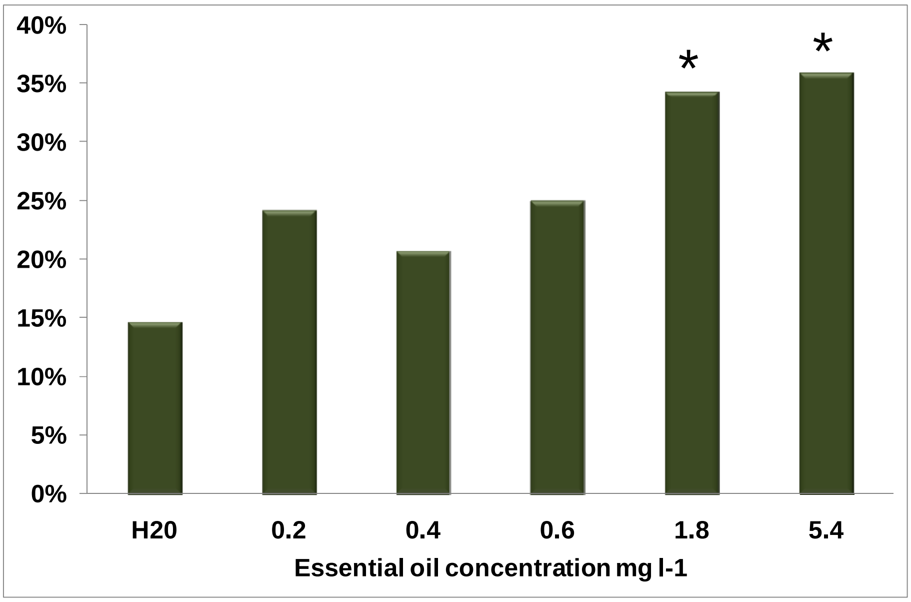


Figure 14 **Comet test on *Vicia faba* cells (%DNA in the Tail) an asterisk show significant differences with P<0.05**



2.7.3 Conclusion

This preliminary screening suggests that the essential oils could be useful as potential bioherbicides as an alternative strategy. The essential oils could avoid the chemical pollution caused by conventional agriculture, reduce or eliminate toxic substances residual in the food and at last improve the nutritional and organoleptic characteristics of the cultivated products (minor nitrate content, greater vitamins content, greater dried substance content) (Sturchio *et al.* 2007).

Additional work is required to determine the mechanism of herbicidal action and to develop a formulation that could be used under open field conditions. Essential oils are in fact highly volatile and their persistence in the open field condition should be improved, as well as it should be investigated the effect on crop species. Further field research is necessary to develop an appropriate technology of essential oil application for inhibiting weed seed germination. Probably loss or interference of mitotic activity might also be responsible for the observed reduction or inhibition of weed seeds germination (Sturchio *et al.* 2007); after the germination there could be some biochemical process or mitotic interference due to the essential oils activity that inhibit the cotyledon growth (Sturchio *et al.* 2007).

2.8 Enzymatic Biotransformation of essential oils

Phytotoxicity and genotoxicity results (Chpaters 2.5, 2.6, 2.7) highlighted the possible use of essential oils as bioherbicides. Unfortunately trials underlined the low persistence of the essential oils. At first, this feature has been to ascribe to the high volatility of essential oils. In this study, developed in collaboration with the Unit of Medicinal and Aromatic Plants in the Newe Ya'ar Research Center of the Israeli Agriculture Research Organization, the biotransformation of essential oils as main motivation is described. For this reason, it was tested the biotransformation occurring to different compounds present in the citronella essential oil once applied to inhibit wheat seeds germination. In particular, the fate of enantiomers of these compounds was investigated. Enantiomers of a same compound are a pair of optical isomers that are mirror images of each other and have identical physical properties, but often they do have different chemical properties.

2.8.1 Materials and Methods

<u>Compounds tested</u>*:* The essential oils tested were (*R*)-(+)-citronellal, (*S*)-(-)-citronellal, (*R*) -(+)-citronellol, (*S*)-(-)-citronellol, (*R*)-(+)-citronellic acid, (*S*)-(-)-citronellic acid. The compounds were purchased by Sigma Aldrich. All the compounds were certified. The compositions of the compounds were subsequently identified using gas chromatography and GC-MS analysis previously described by Ravid *et al.* (1997).

<u>Bioassay of Inhibition Induced by Essential Oils</u>*:* Seeds of wheat *(Triticum aestivum* L. cv Dariel), were germinated in Petri dishes (6 cm diam.) on three layers of filter paper (Whatman No. 3) wetted with 2.5 ml of distilled water. Petri dishes containing 20 seeds each, were incubated in the dark at 27°C. To test the inhibitory effect of each compound, a known amount of it was loaded (using a calibrated glass micro capillary) on a piece of filter paper attached (by double-sided adhesive tape) to the inner side of the cover of the Petri dish. Up to 4 µl of compound and blank controls were tested with this procedure. One micro liter of essential oil per Petri dish is equivalent to 40 nl/ml. Each amount was tested in three replicates. After incubation, the numbers of germinated seeds were counted and the length of each radicle was measured. The amount of essential oil required to cause 50% inhibition of germination was determined by

interpolation from the curve obtained by plotting the means of the replicates against the amount of essential oil applied.

<u>Bioconversion of compounds</u>: Wheat seeds, *Triticum aestivum* L. were germinated in glass vials, 25 ml, on three layers of filter paper, (Whatman No. 1) wetted with 1.5 ml distilled water. Vials containing 20 seeds each, were incubated at 27°C in the dark. Different paths of the bioconversion were tested; in shortage of O_2 (the vials were closed hermetically), in abundance of O_2 (opening and closing the vials every 3 hours) and with N_2. To test the bioconversion of the each compound, a known amount of every examined monoterpne was loaded (using a calibrated glass micro capillary) on a piece of filter paper, which was attached to the inner side of the cover of the vial. Amounts of up to 4 µl of compound were applied in this way. The compound per vial (1 µl) was equivalent to 40 nl/ml. Experiments were repeated in five replicates. After 24 h seeds were extracted with methyl *tert*-butyl ether (MTBE) and the embryo and the endosperm were separate.

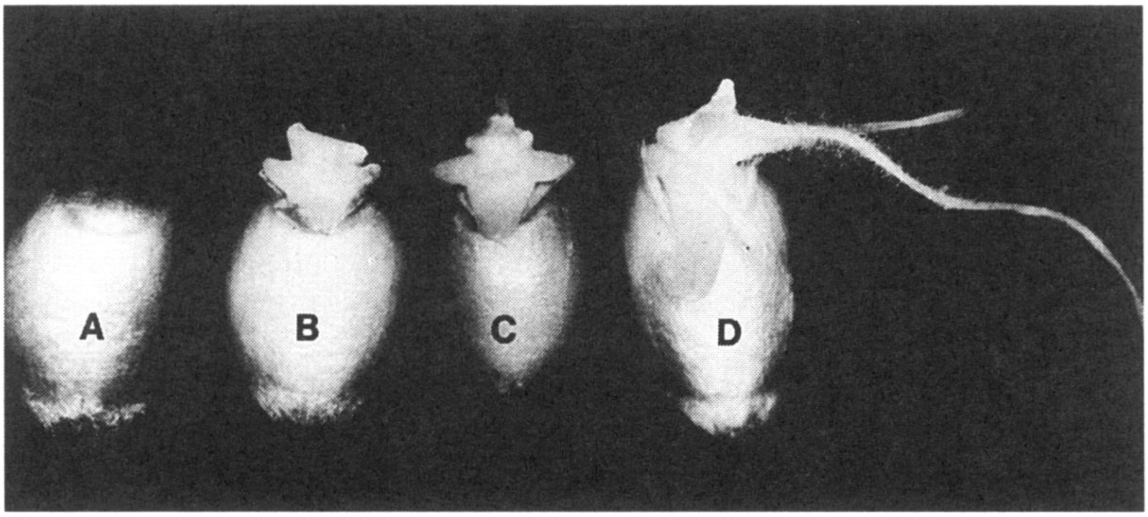
<u>Determination of compounds in the Seed</u>: The examined monoterpenes were: (*R*)-(+)-citronellal, (*S*)-(-)-citronellal, (*R*)-(+)-citronellol, (*S*)-(-)-citronellol, (*R*)-(+)-citronellic acid, (*S*)-(-)-citronellic acid. Seeds were washed by mild shaking for five sec in distilled water. Soon after an extraction with MTBE, containing 10 µg/ml iso-butylbenzene, as an internal standard, was performed for 24 h with gentle shaking at room temperature. The samples were analyzed using an HP-GCD apparatus equipped with a HP-5 MS (30 m x 0.25 mm) fused silica capillary column. Helium was used as the carrier gas. Injection temperature was 250°C, the transfer line temperature was 280°C. Column conditions were: 70°C for 2 min, followed by 4°C /min to 200°C. The components were identified by co-injection with authentic samples and by comparison of the EI-MS obtained from computerized libraries.

2.8.2 Results and Discussion

<u>Essential Oils and Germination Inhibition</u>: The response of wheat seeds to each compound is shown in Figure 15. As describe by Dudai *et al.* (1999) a radical emergence as in Figure 15C were considered to have germinated, Figure 15D shows a control seed not exposed to oil. Seeds with a protrusion of non differentiated cells without radical emergence were considered not germinated (Figure 15B). The radicle of

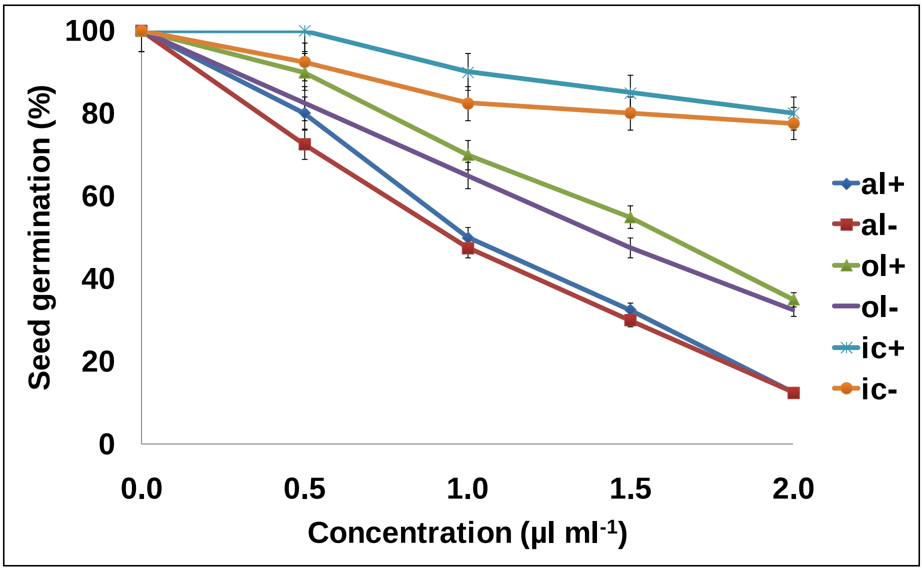
germinated seeds exposed to essential oil (Figure 15C) was shorter than that of control seeds (Figure 15D).

Figure 15 **Response of wheat seed to essential oil. Photograph take 48 hr after sowing. A-C show different responses to the essential oil: (A) seed did not germinate, (B) incomplete germination with lack of radical emergence, (C) seed did germinate, (D) a control seed, not exposed to oil.**



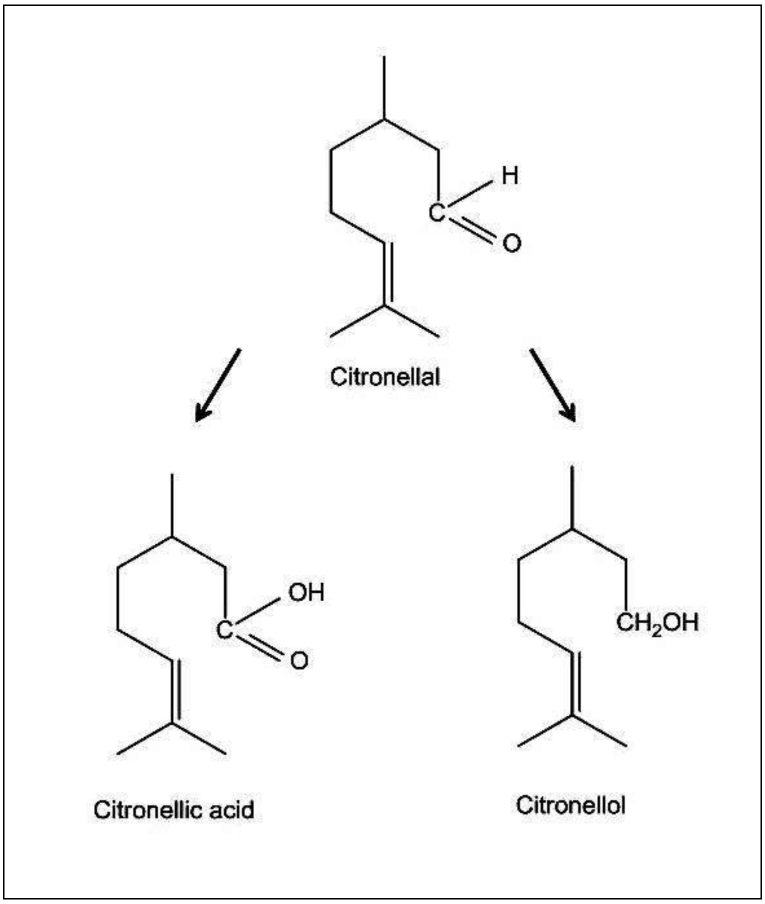
The effect of each compound on germination of wheat seeds is shown in Figure 16.

Figure 16 **Effect of enantiomers (+ and -) on the wheat seed germination; al, ol, ic, represent citronellal, citronellol and citronellic acid, respectively. The bars indicate LSD ($P \leq 0.05$)**



Since compounds inhibit both germination and growth (Figure 15), the effect of each compound on wheat seedlings was studied. The amount of essential oil required to cause 50% inhibition of germination was determined as previous did Dudai *et al.* (1999) by interpolation from the curve obtained by plotting the means of the replicates against the amount of essential oil applied. Fifty percent inhibition of growth of radicles occurred at 0.96 µl/ml and 1.00 µl/ml of (*S*)-(-)-citronellal and (*R*)-(+)-citronellal respectively compared with 1.42 µl/ml and 1.62 µl/ml required for 50% inhibition of germination of (*S*)-(-)-citronellol and (*R*)-(+)-citronellol (Figure 16).

Scheme 1 Metabolism of Citronellal



The important feature highlighted from these results is how starting from citronellal the inhibition ability of the compounds decrease considerably through the process of oxidation or reduction (Scheme 1, Figure16).

<u>*Bioconversion of compound:*</u> Seeds were exposed in contact or gaseous phase to citronellol, citronellal or citronellic acid and the amount of the compound and its derivates was determinate in the embryo and endosperm using a gas-chromatographic (GC) analysis combined with mass spectrometry (MS) (data not showed). The compound and its metabolite were detected both in the endosperm and the embryo. When citronellal was supplied to the seeds, GC–MS showed the presence of new compounds in the treated seeds. In particular the appearance of citronellol and citronellic acid was observed. Wheat seeds used without disinfection showed derivatives citronellol and citronellic acid due to the combined activity of the seeds and the contaminant microorganisms. Previous studies conducted by Dudai *et al.* (1993, 2000) showed just the enzymes effect. The kinetics of the appearance of the derivatives of citronellal were exanimate (Scheme 1). The transformation occurs both in the embryo and endosperm already at the beginning of imbibitions.

2.8.3 Conclusion

The results presented show that wheat seeds, which do not contain monoterpenes, are able to metabolize them. The monoterpenes and their metabolic derivates are primarily the result of oxidation and reduction, although it is clear that part of the compounds are completely degraded since it was not possible to detect any of the derivates using gas chromatography and mass spectrometry. Dudai *et al.* (2000) indicate that seeds act as a sink for the monoterpenes, thereby increasing loss from the source. It is possible that citronellal was degraded to carbon dioxide or lost in the vapor phase. It was important to determine whether other monoterpenes and related compounds were also metabolized. As can be seen (Scheme 1) the aldehydic monoterpene citronellal was metabolized with the formation of the reduced and oxidized derivatives citronellol and citronellic acid. This suggests the possible existence of non-specific mechanisms, which can result in the oxidation or reduction of aldehydes. In particular the degradation of the essential oils requires the activity of several enzyme systems (Dudai *et al.* 2000). Reductive process might be catalyzed by non specific dehydrogenases (Plapp *et al.* 1993) and oxidation might be due to the presence of cytochrome *P*-450 type enzymes, which today are known to be present in plant tissue and are involved in the biosynthesis of terpenes (Mihaliak *et al.* 1993; Halkier, 1996). Most investigations on allelopathy have characterized the phenomenon but did not consider the mechanism taking place. Quite often the short persistence and the decreasing inhibition ability during time have been attributed to the high volatility of the oils, not taking into consideration the biotransformation during the germination process. In the present study, we have examined the biotransformation, as detoxification process, occurring to citronellal. This process converts it into less aggressive and injurious compounds as demonstrate by their reduced inhibition ability (Figure 16). Our findings demonstrate the potential of essential oils as allelopathic agents and provide an insight into their mechanism of action and persistence.

2.9 Microbial Bioconversion of essential oils

The study, developed in the Unit of Medicinal and Aromatic Plants of the Israeli Agriculture Research Organization (Chapter 2.8), emphasized the enzymatic biotransformation of essential oils during the germination of wheat seeds. Literature review suggested that essential oils in the open field condition could be inactivated by microorganisms. Hence, further research on the transformation of essential oils has been carried out in the Department of Agrobiology and Agrochemicals of the University of Tuscia. The research developed was addressed to isolate and identify microorganisms present on wheat seeds coats and responsible of essential oils bioconversion/degradation.

2.9.1 Materials and Methods

A comparison between microbial populations occurring in the seed coats of an Italian and an Israeli wheat variety were carried out. The Italian wheat variety (*Triticum aestivum* L.) were obtained by the experimental farm of the University of Tuscia, while the Israeli wheat variety (*Triticum aestivum* L. cv Dariel) were obtained by the Unit of Medicinal and Aromatic Plants of the Israeli Agriculture Research Organization. Both seeds assortments were not treated with fungicides.

Microorganism isolation: for each variety, 10 grams of wheat seeds were transferred into a flask of 250 ml with 25 ml of physiological solution (NaCl 0.95% w/v) and incubated at 30°C overnight. An aliquot was extracted and after serial ten-fold dilutions, aliquots were plated onto 10-cm diameter Petri dishes containing a nutrient-rich medium, such as Luria-Bertani agar (LBA) or Tryptic Soy Agar (TSA), in order to obtain single colony. Hence, plates were incubated at 28°C for 24 hours. Colonies showing different color, size and morphology were isolated, and then streaked onto LBA plates until to obtain purified single colony. Purified cultures were stored at room temperature on LBA plates or maintained for long-term storage at −80°C in LB broth supplemented with 20% (v/v) glycerol. Genomic DNA was extracted from bacterial suspension of pure cultures using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purity and the concentration of the DNA template was verified by agarose gel electrophoresis with ethidium bromide staining on a 1.0% (w/v) agarose - 1xTAE (40mM Tris/acetate, 1mM EDTA; pH 8.3) gel.

PCR Amplification: The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been by far the most common housekeeping genetic marker used for a number of reasons. These reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rRNA gene (1,500 bp) is large enough for informatics purposes (Patel, 2001). Bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) with the following primers:

63F, 5′-CAGGCCTAACACATGCAAGTC-3′

1389R, 5′-ACGGGCGGTGTGTACAAG-3′

with the thermal profile described by Bertin *et al*. (2006).

DNA Sequencing and Phylogenetic Analysis: Genes, 16S rRNA, were amplified as described before from genomic DNA samples obtained from 20 single colonies from Italian and 20 from Israeli seeds solution.

Unlabeled PCR products, purified as described above, were cloned using the pGEM-T Easy Vector System (Promega), according to the manufacturer's instructions. Plasmid DNA from randomly selected clones was purified, using a Wizard Plus SV Mini Prep kit (Promega), and subjected to cycle sequencing using M13 uni & reverse sequencing primers and the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The DNA sequences were bidirectionally resolved on an ABI Prism 310 in a sequencing mode. Nucleotide sequences were assembled and compared with the sequences in the RDP database (Ribosomal Database Project) to identify the closest relatives.

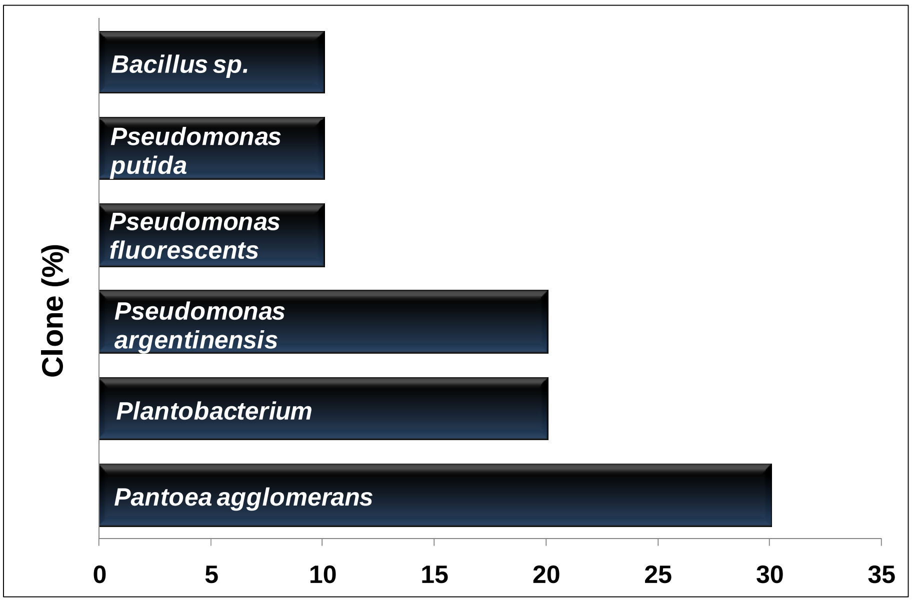
Analysis of terminal restriction fragment length polymor-phism (T-RFLP): Community fingerprinting of seed coat samples by T-RFLP was carried out as described by Bertin *et al*. (2006). The 16S rDNA fragments were amplified by PCR from total DNA extracts with the primer pair 63F-1389R in which the 63F primer was labeled with a fluorescent nucleotide derivative. Fluorescently labeled PCR products were purified with QIAquick PCR purification kit (Qiagen), and 100 ng were digested with 10 U of the restriction enzyme *Hha*I (Invitrogen, Italia) for at least 4 hours at 37°C. The digested products (2 μl) were mixed with 19.5 μl of deionized formamide and 0.5 μl of ROX-labeled GS500 internal size standard (Applied Biosystems) and denaturized for 5 min at 95°C before capillary electrophoresis on ABI Prism 310 Genetic Analyzer (Applied Biosystems). Electropherogram analysis was performed using GeneScan Analysis 3.1 software using the local southern size calling method.

Terminal restriction fragment patterns of 16S rRNA genes derived from total community DNA were compared with the T-RFLP profiles of individual clones and cultures for accurate identification.

2.9.2 *Results and Discussion*

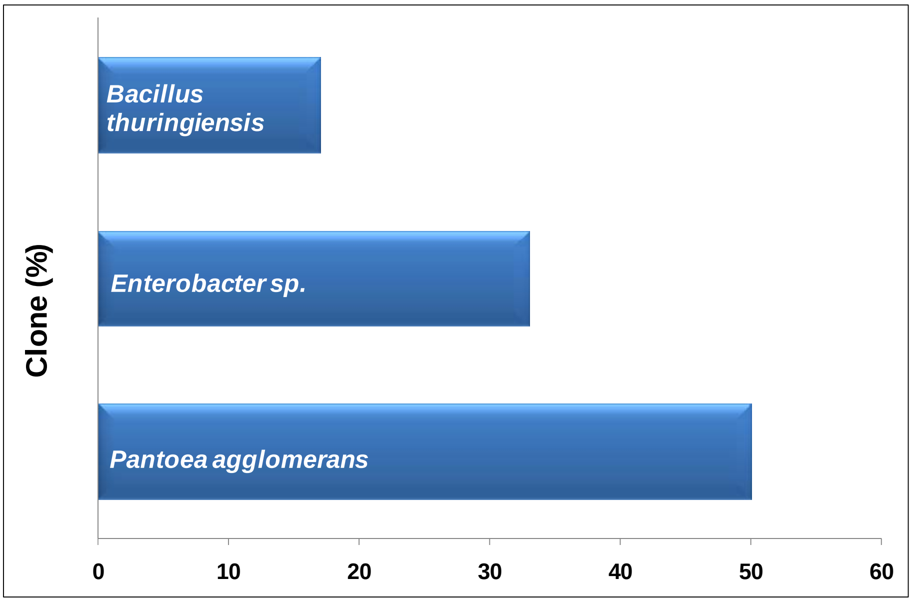
The 1500-bp nucleotide sequences of the 16S rRNA gene from 20 colonies from Italian and Israeli seed solutions were aligned and compared. The results obtained highlighted that the bacterial community on Italian seeds is characterized by a higher heterogeneity compared to Israeli one. The RDP Sequence Match tool was used to find the closest relatives in the RDP database for each sequence and, relying on this phylogenetic analysis, it was concluded that in the Italian seeds there are at least six bacterial species instead of the three found in the Israeli seeds (Figures 17 – 18).

Figure 17 **Clone distribution based on the sequences of 16S rRNA genes amplified in samples from Italian seeds**



Even if the analysis of the bacterial community of the two seed varieties showed substantial differences, in both communities *Pantoea agglomerans* resulted being the predominant bacterial specie. The T-RFLP analysis, using to compare microbial communities and presumptively identify abundant members, confirmed that *P. agglomerans* is the main bacterial specie for both seeds varieties (data not showed). In particular *P. agglomerans* represents 30% and 50% of the total bacterial community find in the Italian and Israeli seed coats respectively.

Figure 18 **Clone distribution based on the sequences of 16S rRNA genes amplified in samples from Israeli seeds**



2.9.3 Conclusion

Bibliography indicates that microorganisms take part in the detoxification process of monoterpenes (Dudai *et al.* 2000). Results of the enzymatic bioconversion trials indicate that the short persistence of the essential oils is due to their degradation instead of volatility. In fact, citronellal is converted into less aggressive and injurious compounds as previously demonstrated by its reduced inhibition ability (chapter 2.8). Park *et al.* (2003, 2004) indicated that *P. agglomerans* (formerly *Enterobacter agglomerans*) is responsible in bioconversion of citronellal. If this study is confirmed, the presence of *P.*

agglomerans on the wheat seed coats could explain the short persistence of essential oils during seeds germination due to bacterial degradation as already did for the enzymatic biotransformation.

DIFFUSION PHASE

3.1 How E-Learning can improve knowledge diffusion

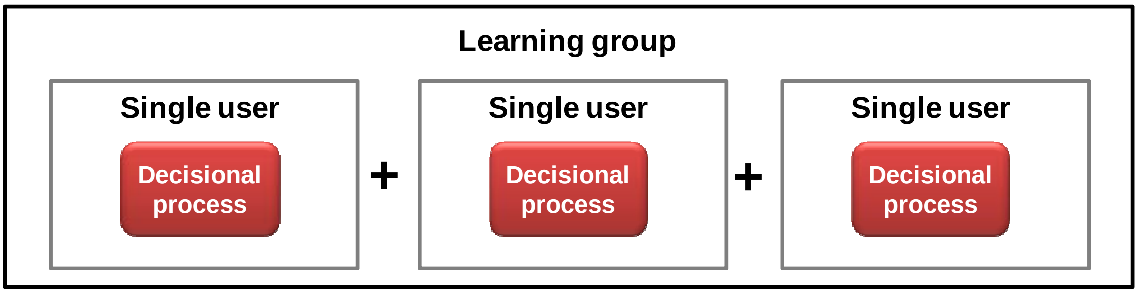
Internet has increased the opportunity for flexible approaches to information and learning. Despite common belief, Internet-based information and instruction are by no means the “magic bullet” able to guarantee a rich learning environment (Caplan, 2004). Research continues to confirm that there is no significant difference among student outcomes based on mode of course delivery (Russell, 1999). As far as E-Learning is just considered a surrogate of information and learning material made available on electronic device, this sentence can be accepted. Differently we must keep in mind that E-Learning plays a pivotal role in the development of constructivism vision generated by the social action and participatory media. E-Learning, indeed, represents a tool for knowledge creation and diffusion, not just in the academic world but in the social framework and for all the average citizen. Through community and participatory media, E-Learning has the advantage of communicate and inform people in different geographical area and with different knowledge background without discrimination. The easy accessibility and availability represents its distinguishing quality. Once given the accessibility and freedom to share information at all the level and to all the people with different background, E-Learning becomes a tool to compare and improve knowledge conveying the common intent of actively involving people who are the ‘subjects’ of development in shaping the process. People utilizing E-Learning develop a compensatory adaptation to problem solving and learning environment which increase the cognitive effort required to communicate and create new ideas and knowledge (Kock, 2008). In this frame E-Learning plays a crucial social role to improve knowledge construction and diffusion.

3.2 Social activity as requirement for knowledge construction and diffusion

Social constructivism is based on the idea of learning as a social activity rather than individual (Lave & Wenger, 1991; Jonassen, 1998). Social constructivism is based on the idea of learners, building existing knowledge within a context of social and collaborative learning. Each single individual user is part of a learning group (Figure

19). In this way it is recognized the role that society plays in building knowledge through joint construction with other learners and where knowledge is a continuous process and collaborative modernization of experience (Garrison & Archer, 2000). Hence fundamental to all learning process is the ability to communicate and interact.

Figure 19 Learning Group

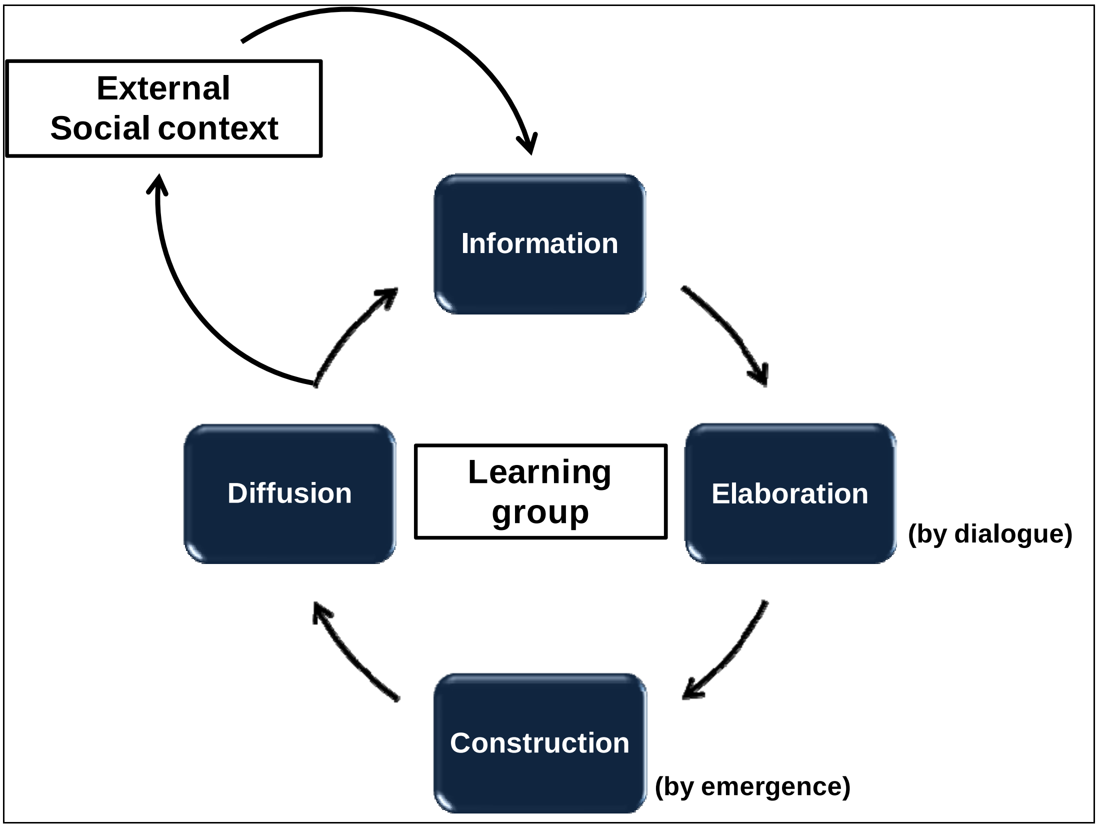


This approach suggests that learners actively construct knowledge by building and sharing prior knowledge and experience. For this reason, an E-learning environment is identified by six pivotal points as classified by the Joint Information Systems Committee study (2004):

- Connectivity – access to information is available on a global scale.
- Flexibility – learning can take place any time, any place.
- Interactivity – assessment of learning can be immediate and autonomous.
- Collaboration – use of discussion tools can support collaborative learning.
- Extended opportunities – e-content can reinforce and extend topic-based learning.
- Motivation – multimedia resources can make learning fun.

All these features, which can be found either in the synchronous or in the asynchronous E-Learning module, can boost and improve communication and interaction between learners. In synchronous discussions, participants log on at the same time and participate in "real-time" chat sessions, usually text-based, although audio and video software is available. Asynchronous communication methods include email, electronic bulletin boards, and online discussion forums that participants can access at any time. Between the two forms, asynchronous discussion forums, that people can access at any time, represent the real innovation and backbone of the E-Learning platform. Online forums are, in fact, used to provide the context for participants to interact and build their knowledge of discipline areas. Hence internet provides an opportunity to create learning environments and reliable experiences where average citizen can explore and co-create knowledge. While internet gives citizen free access to huge stores of information, E-Learning is structured and designed to support learners in the task of turning this information into knowledge. It is possible to identify four stages of social knowledge creation and diffusion and representing them as a constant learning cycle (Figure 20).

Figure 20 Social knowledge creation



The constant learning cycle is addressed at social knowledge creation through:

- Information – the information or knowledge generate by the decisional process of each single user in a certain context, represent the starting point for the social constructivism.
- Elaboration – the information are elaborated by means of social contact with other peoples' concepts and knowledge. This involves a *dialogue*, an interaction between the user's pre-existing framework of understanding. The dialogue,

indeed, refers to the testing and tuning of elaboration through discussion, such as forum, chat, electronic bulletin boards, e–mails.

- Construction – is the process of the "emergence" of new knowledge by building up and combining concepts.
- Diffusion – the outcome of the construction is the contribution to the diffusion of new ideas and knowledge.

The leading process to create knowledge, is the decisional process (see chapter 1.3). Anyway the process is made effective and sustainable through the information exchange between stakeholders, hence the community become relevant. It must be appreciated that the social aspects of learning bring into focus the extent to which an individual user is part of a learning group, and the extent to which that group can be considered as an emerging community of practice into society (Mayes, 2002).

3.3 From construction to diffusion of knowledge in Agroecology – a practical application

The European Action Plan for Organic Food and Farming (EC, 2004) has identified the need for actions supporting the training and education of all stakeholders related to organic agriculture, covering aspects related to production, processing, marketing and their benefits.

Among plant production features, weed control still represents the bottle neck of the organic agriculture production system. Weed management in organic agriculture, in fact, due to the ban of synthetic herbicides, must involve the use of many techniques and strategies (Lampkin, 1990). Mechanical and agronomical features are frequently implemented together, trying to give to the crop a competitive advantage on weed. These approaches are often economically expensive and are not always efficacy. Among organic techniques, the use of natural defense such as allelopathic compounds could be a feasible approach to weed management (see chapters 2.1, 2.2, 2.3). Allelopathic compounds, are secondary metabolites that are not directly involved in the normal growth, development or reproduction of organisms. The function or importance of these compounds to the organism is usually of ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition (Molisch, 1937; Rice, 1984; Duke and Abbas, 1995; Dukes *et al*. 1996).

Hence ‘From construction to diffusion of knowledge in Agroecology’ aims at improving the organic weed management research sector, involving students, researchers, private Institutions and Universities. For this reason, the work of the thesis was made up and developed in two phases: a *construction phase* and a *diffusion phase*. The construction phase dealt with experimental trials on the use of essential oil as bioherbicides. Essential oils of lavender, cinnamon, peppermint and citronella, were tested in a scale up system on weed and crop seeds. The experiment started from a situation of controlled conditions (germination chamber). Temperature, humidity, light, and the utilization of inert substrate, such as filter paper into Petri dishes, allowed to check just the phytotoxic effect of compounds tested. In the second instance, moving to semi controlled condition (green house), light, humidity and the substrate utilized (loamy soil), made the experiment much closer to the natural environment, characterized by variability and biological activity, allowing to assess the real potentiality of the essential oils. At the same time, experiments on the genotoxicity, bioconversion and biotransformation of essential oils were carried out. The genotoxicity test asserts that essential oils can be used as bioherbicides. The trials on bioconversion and biotransformation emphasized the effective control of essential oils as bioherbicides in controlled conditions, while, environment factors and enzymatic and microorganisms transformations, make their application in the open field more difficult. It was concluded that further research is necessary to develop an appropriate technology of essential oils application. So far, the diffusion phase could contribute to share a common knowledge and to develop participatory solution to problems faced during the research. In fact, building up an E-Learning platform is the aim of the diffusion phase. Besides the results achieved, to share information and experience about weed control with people involved in the same topic and research is a rational prospect to improve and discover new and effective solutions. In this way, it should be possible to define a common action in a learning group to validate the research results by implementing activities with external and potential users (i.e. external networks of teachers and agricultural universities). In this light, the purpose could be the involvement of the existing networks of scholars as learning groups, for instance Organic.Edunet as well as ENOAT (European Network of Organic Agriculture Teachers) to create a dynamic community of students, researchers and stakeholders focused on weed control in organic farming. The platform could overwhelm the research and information gap by means of learning repositories, forums, chats, in order to suggest solution to the research. In this way, there could be the opportunity to develop together a same research line in different countries with the support of national and international specialists and stakeholders. This target fulfills the need for actions to support the training, education and application of new knowledge in organic agriculture either at a national or international level and dimension.

3.3.1 Organic.Edunet

Organic.Edunet is a big Web portal that aims to facilitate access, usage and exploitation of digital educational content related to Organic Agriculture and Agroecology. It identifies the need to increase consumer awareness and education about organic agriculture, as well as to provide education for all actors involved (e.g. farmers, processors, agricultural experts, etc.).

Organic.Edunet focuses on two particular dimensions:

1. To familiarize children with the concepts and benefits of Organic Agriculture and Agroecology, through formal educational systems, starting from compulsory and secondary education;
2. To educate young agricultural experts (including agricultural engineers, agricultural economists, extension officers, etc.) about the methods and practices of Organic Agriculture and Agroecology, through formal educational systems of higher education (i.e. agricultural universities).

Both groups (future consumers and future agricultural experts) constitute user groups of high importance. Children constitute tomorrow's consumers, and they have to be properly approached and educated so that their nutritional, as well as their ecological and environmental cultures are developed. Students of agricultural universities constitute tomorrow's agricultural professionals. They are expected to guide farmers through the adoption of Organic Agriculture and Agroecology principles, or to serve themselves as the next generation of farmers/producers. Therefore, these two user groups have to be carefully approached through relevant educational programs, and appropriately supported by publicly-available, quality, and multilingual educational content.

The main objectives of the Organic.Edunet project are:

- To support stakeholders producing content about Organic Agriculture and Agroecology to publish it in an online federation of learning repositories, described according to multilingual, standard-complying metadata.
- To deploy a multilingual online environment (the Organic.Edunet Web portal) that will facilitate end-users' search, retrieval, access and use of the content in the learning repositories.
- To study educational scenarios that will introduce the use of the Organic.Edunet Web portal and the content in the repositories, in order to support teaching of the relevant topics, in high-schools and agricultural universities.
- To evaluate project results in the context of both focused pilot trials and open validation events which will take place in various European schools and universities.
- To create organizational structures that will reinforce the cooperation of stakeholders in this particular content area and will support the sustainability of project results.

3.3.2 ENOAT

ENOAT (European Network of Organic Agriculture Teachers), is a network of University teachers with the goals to coordinate courses on organic production, processing and marketing, and to facilitate the exchange of students and teachers. Specific objectives include:

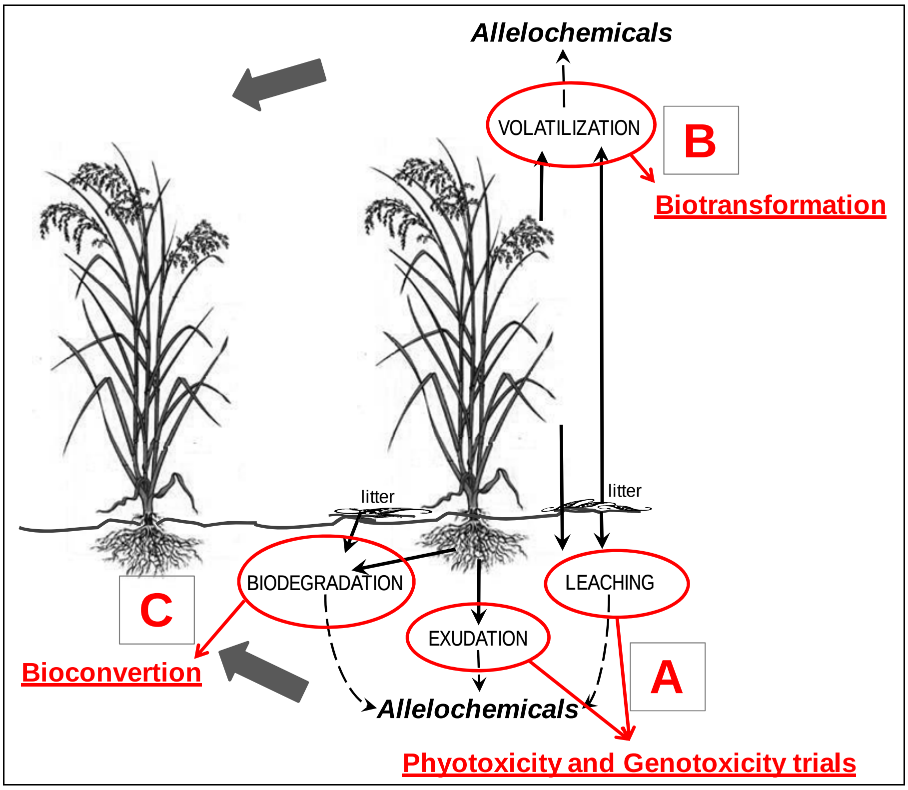
- To promote international collaboration among university teachers and students of organic agriculture and prepare the students for teamwork across EU and pre-accession countries.
- To discuss and coordinate curricula and courses in organic agriculture with EU partners and representatives from pre-accession countries that currently do not have university programs in organic agriculture.
- To improve teaching quality in the subject of organic or ecological agriculture in EU member states and support university teachers in pre-accession states to enhance their knowledge of and teaching capacities in organic agriculture.

- To get first-hand knowledge of organic agricultural practices in various member and pre-accession states through annual meetings and summer courses rotating among network partners.
- To develop course materials for teaching principles of organic agriculture.

3.4 From construction to diffusion of knowledge in Agroecology – the weed control knowledge base

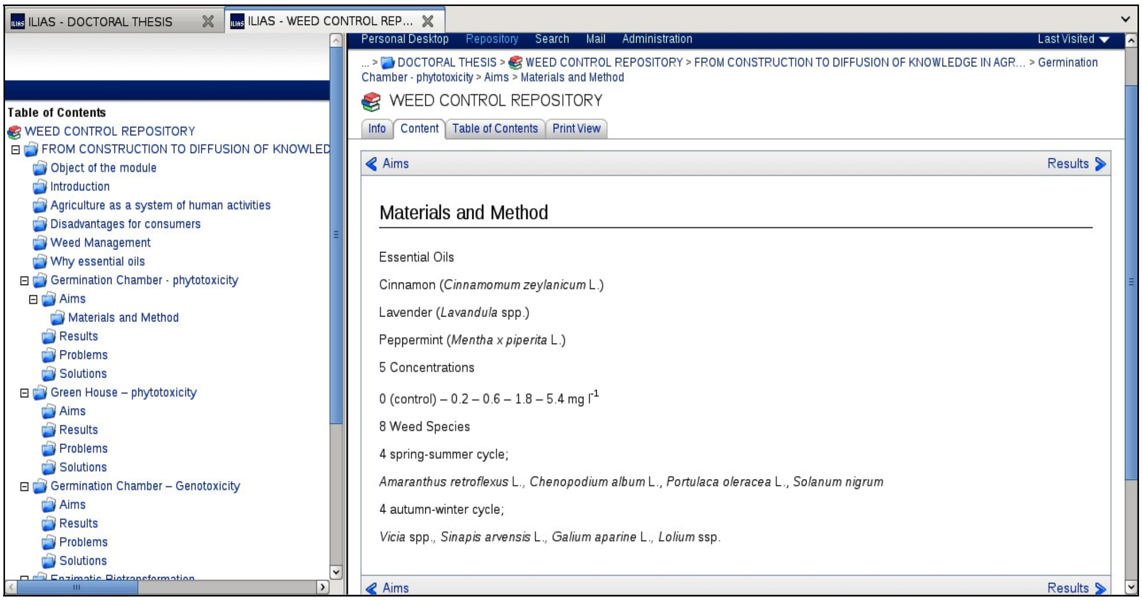
In order to structure and organize a weed control repository (Figure 22), it is fundamental taking into consideration not just the result acquired, but rather the problem faced during the research. In fact, the E-Learning platform represents an important "tool" based on participatory media (subchapter 1.1.2) for knowledge creation by means of learning group suggestions in order to create new knowledge through dialogue and debate The work of this thesis has been developed following a scale up process to create new knowledge on the use of essential oils as bioherbicides. All experiments started from knowledge of previous trials. The research carried out produced new knowledge but also new problems and the need for much more effort to find out a solutions. At the same time, even if good results had been achieved, the constructive work of an E-Learning platform and the presence of a learning group could improve their application and improvement. It has already been described how E-Learning can improve the decisional process (chapter 1.3) and how the single user action can develop into social knowledge creation (chapter 3.2). So in this chapter is described where and how the research has been developed, improved, and where the learning group (Organic.Edunet and/or ENOAT) could operate constructively giving feedback and feed forward. First of all, weed control repository is addressed to students, researchers and teachers involved in organic agriculture. Particularly it is addressed to all stakeholders involved in weed control in organic farming. Hence, the level of the information, due to the target group knowledge and background, facilitates the kind and level of background that will be shared. The use of essential oils as allelochemicals represents, indeed, just a peculiar aspect of weed management, relying on natural defenses and competitive ability of crop species and weeds. The aim of the repository is to improve and facilitate the development of new research and/or giving new knowledge to informed groups of stakeholders. The information input has been developed taking into consideration the release of allelochemicals by the donor to the target plant. The figure (21) represents and summarizes the starting point of the trials developed. The phyotoxicity and genotoxicity trials (germination chamber and green house), indeed, reproduced what happens on crop and weed seeds put into contact with secondary metabolites, thus allelochemicals *exudates* or *leaches* (Figure 21 link A). Biotransformation of essential oils by enzymatic effect represents the effect of the *volatilization* (Figure 21 link B) of allelochemicals interfering in gaseous phase with seeds germination. Bioconversion represents the *biodegradation* (Figure 21 link C) of essential oils by microorganisms. Last but not least, the phytotoxicity and genotoxicity effects, in general, represent the interference of essential oils on the germination of various seeds species.

Figure 21 Release of allelochemicals (modified from Devi *et al.* 1997), as structure of the research developed



All these features has been developed and investigated in order to give an answer to the allelochemicals action and diffusion in the environment. The contribution of this thesis work to the weed control topic is the collection of a series of trials developed in a scale up system, thus, giving continuity to the knowledge construction phase. Hence, the user will be put into condition to learn and investigate all different aspects of the phenomenon into a single case study (Figure 22). In this way, following all the steps, it will be possible to analyze the rational of the research carried out, identify problems and try to find solutions in a participatory way. Indeed at the end of the repository, the user should be able to interact in a critic and constructive way with and within the platform and the learning group, identifying and suggesting alternative solutions to the *modus operandi* developed. For facilitating this process, for each trial are below highlighted aims, results and problems faced during their development. This kind of basic information is reported in the content list of the repository (Figure 22). In this way it is possible to understand how problems have been arisen and partially solved during the scale up process.

Figure 22 Repository on the research developed, as a case study of “From construction to diffusion of knowledge on agroecology”



3.4.1 Germination Chamber – Phytotoxicity of essential oils

Aim of the research was the screening of essential oils extract from aromatic plants, as possible bioherbicides. For doing that, trials have been developed in a germination chamber. The controlled conditions of temperature, humidity and light, as well as the utilization of an inert substrate, such as filter paper into Petri dishes, allowed to check only the phytotoxic effect of compounds tested. In particular, were tested essential oil as a mixture of compounds, and not each single compound. The allelopathic inhibition under natural conditions, in fact, is the result of the combined effect of several compounds. The essential oils of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) were tested on seed germination of seven common weed species from the Mediterranean environment: *Amaranthus retroflexus* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Chenopodium album* L., *Sinapis arvensis* L., *Lolium* spp., and *Vicia sativa* L.. Results from the first screening, proof the possible use of the essential oils in controlled condition, but their effect in a natural environment characterized by biological activity were to be investigated. Hence, following a scale up process, a Green House trial was set up.

3.4.2 Green House – Phytotoxicity of essential oils

After the results obtained in the germination chamber, the research moved to semi controlled conditions. Hence the experiment was carried out in a green house. Light, humidity and the substrate utilized (loamy soil), made the experiment close to the natural conditions characterized by variability and biological activity. The experiment was designed to check the inhibition ability exerted by the essential oils of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) on seed germination of common weeds species of the Mediterranean environment: *Amaranthus retroflexus* L., *Sinapis arvensis* L., *Lolium* spp.. As recorded in the trial conduced in the germination chamber, it was confirmed the possible use of essential oils as bioherbicide. Anyway, the problem faced during the green house trial was the short persistence of the essential oil. The first suggestion to this problem was the volatilization of oils. Besides the persistence, as no dose able to control totally the seeds germination was individuated, was supposed the inactivation of essential in the environment. Furthermore, it was investigate the possible inhibition activity of essential oils on crop species as well. Hence further trials on the phytotoxicity were conduced, as well as a genotoxicity test to investigate the possible damage and mechanism of action of the essential oils.

3.4.3 Germination Chamber – Genotoxicity of essential oils

After having conduced trials in controlled and semi controlled environment of essential oils on weed seeds germination, a test on a crop specie were carried out. Furthermore the genotoxicity feature was investigated. Essential oils, in fact, are generally regarded as safe by the United States of America Food and Drug Administration (FDA). Hence, in this research the essential oil of lavender (*Lavandula spp*.) was tested on *Vicia faba* seeds. The test suggested the mechanism of action of essential oils, demonstrating the phytotoxic effect even on a crop species (*Vicia faba*) and its genotoxicity. Yet, a dose-dependent relation between oil concentrations and genotoxic effect was not found. This suggests a transformation of oils due to the substrate utilized for the germination as well as an environmental interference, instead of a volatilization process. Hence, the following step of the research has been a study on the conversion of the main compounds of essential oils by enzymatic and microbiological activity.

3.4.4 Enzimatic Biotransformation of essential oils

Phytotoxicity and genotoxicity results highlighted the possible use of essential oils as bioherbicides, even if some external interference with oils were recorded. Previous trials underlined the low persistence of the essential oils. At first, this feature was ascribed to the high volatilization of essential oils. In this trial, instead, is described the biotransformation of essential oils as main motivation of short persistence. For this reason it was tested the biotransformation occurring to different compounds present in the citronella essential oil once applied to inhibit wheat seeds germination. In particular, was investigated the fate of single compounds, such as enantiomers. Enantiomers of a same compound are a pair of optical isomers that are mirror images of each other and have identical physical properties, but often they do have different chemical properties. As hypothesized, the essential oil had phytotoxic effect on crop species such as wheat. Furthermore, the short persistence has been identified a cause of biotransformation of the essential oils. In fact, during the transformation for enzymatic activity, the compound is converted for a detoxification process in a less harmful compound. The process develops in the seed, but the environment has effect as well. Hence, microorganisms were investigated as responsible of further conversion of essential oils.

3.4.5 Microbial Bioconversion of essential oils

The study on the enzymatic biotransformation of essential oils during the germination of wheat seeds highlighted the possible interference of others variables as well. Literature review suggested that essential oils in the open field condition could be inactivated by microorganisms. Hence, research on the transformation of essential oils due to microorganism were developed. In particular microorganisms were isolated and identified. Their presence on wheat seeds coats could improve essential oils bioconversion.

3.5 Conclusion

Trails developed in a scale up process confirm the possible use of essential oils as bioherbicides. The main features of achievement or failure have been identified. With an E-Learning platform it is possible to share a knowledge base for further development of “the possible use of essential oils as bioherbicides”. By mean of a repository, these results can be shared and evaluated within a learning group of specialized stakeholders in organic farming and/or in the social context. Debate and dialogue, will contribute to constructing new knowledge. What is important to highlight is that the knowledge creation (Figure 18) is a cycle. It starts from an information becoming step by step knowledge to be diffused. At the end of the process, usually, once achieved new knowledge, the cycle restarts. The cycle restarts from the last information/knowledge diffused. The process is equivalent to a scale up system, in which each phase starts from the results of previous experiments in a continuous and never ending improvement. In this way, the learning group makes the process even more effective by the combined activity of all users. The decisional process is improved by E-Learning (Figure 2). Already in the interface external context/internal context, E-Learning provides much more accessibility and availability of information. In fact, there are a wider range of information in the electronic network than in conventional networks. For instance, in utilizing a repository about the allelopathic effect of natural substances like that illustrated in this thesis work, there will be the opportunity to have a rich picture of the research developed and results achieved. These information represents the input for the internal context where the interpretation is facilitated by the E-Learning platform and learning group (i.e. Organic.edunet or ENOAT), which give birth to a community aimed at building up knowledge.

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